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High protein meals may benefit fat oxidation and energy expenditure in individuals with higher body fat

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Conclusion: High protein intakes may ameliorate an obesity induced decline in fat oxidation.

Key words: energy expenditure, body composition, substrate utilisation, obesity.

Introduction

There is some evidence that a diet high in protein may promote weight loss[1-3]. Higher dietary protein intakes (>25% of energy) have been reported to have a higher thermogenic effect. Studies investigating the effects of high protein diets on thermogenesis have generally included subjects homogeneous in body composition[4-12]. Where both normal and obese subjects have been recruited results have either been divided into the two body composition groups[13] or presented in a format which did not adjust for body composition differences in the study population[14,15]. More recently Labayen and colleagues investigated differences in energy expenditure and substrate utilisation in obese and lean women consuming high protein and control meals as a liquid supplement[16]. They reported no significant differences in thermogenesis between the protein and control meals when body composition was included as a covariate. This results suggests that body composition (including both the fat and fat free mass) must be adjusted for in analyses where samples are heterogeneous in body composition. Fat oxidation was reported to be improved in the obese subjects, following the high protein supplement in that study.

Fat oxidation has been shown to be impaired in obese and post-obese subjects[17,18] so dietary strategies which improve fat oxidation maybe particularly beneficial. As insulin resistance has been shown to be negatively associated with fasting fat oxidation and positively associated with post prandial fat oxidation in obese subjects[19] dietary strategies which displace fat in the diet maybe particularly beneficial for treatment of metabolic syndrome.

In the context of a meal, the aim of the present study was to test the effects of a realistic increase in dietary protein on acute energy expenditure (EE) and substrate oxidation in a group of healthy individuals varying in body composition. As lower GI foods have been

reported to increase fat oxidation[20], the protein enriched meals used in this study contained either predominantly low GI or predominantly high GI sources of carbohydrate. Thus a secondary aim of this research was to determine if incorporating low GI foods in a high protein diet would enhance any thermogenic effects.

Methods

Recruitment and ethics

Healthy adult subjects were recruited through advertisement. Exclusion criteria were signs or symptoms of chronic disease, or taking any medication known to affect metabolic rate. All subjects gave written informed consent to participate in the study and the study was approved by the University of Wollongong/Illawarra Area Health Service Ethics Committee. All measurements were conducted at the University of Wollongong Room Calorimetry facility and associated Nutrition Laboratories.

Experimental Protocol

The study was an acute randomised crossover design. Subjects attended a prestudy screening visit to familiarise the subjects with the calorimeter facility and to ascertain they were willing to consume the study foods. Subjects were instructed to consume their usual diet and avoid strenuous activity for at least 24 hours prior to the study.

Subjects then attended three study visits one month apart for the women and at least one week apart for the men. Subjects arrived fasting on each occasion, were asked to empty their bladder and then had their body composition and weight assessed before entering the chamber for an 8 hour stay. Gas measurements commenced between 8 and 9 am and ended 8 hours

later. Breakfast was provided 30 minutes after entering the chamber and lunch was served 210 minutes after the breakfast meal. Subjects engaged in sedentary activity (using the computer, watching television) between meal times. Subjects kept an activity diary in the chamber so they were able to replicate their activity on each study visit. Urine was collected during the stay.

Test meals

The energy values and macronutrient composition of the meals used was calculated using the Foodworks nutrient analysis software program (version 3, 2002; Xyris Software, Brisbane, Australia) using the Australian nutrient database AUSNUT. The meals provided and the macronutrient breakdown are outlined in Appendix 1. The meals differed in the amount of protein and the carbohydrate types provided. The caloric intake of each of the diet was designed to be similar in energy, fat calories and type and the amount of calcium provided.

Body composition

Weight was measured using electronic scales (Tanita TBF-622, Tanita Corporation of Japan, Tokyo Japan) and body composition was estimated using a tetrapolar bioelectrical impedance analyser (Bodystat 1500, Bodystat Ltd, Douglas, IM USA). We have previously validated this analyser for use in our laboratory by comparison with dual energy x-ray absorptiometry[21]. The Intra-class correlation for absolute agreement of this instrument for the four measurements (screening and the three study visits) in the 18 subjects who participated in the study was 0.966 indicating good reliability.

Indirect Calorimetry

Oxygen consumption and carbon dioxide production were measured in the whole room calorimeter facility at the University of Wollongong. The facility consists of two separate air-tight, ventilated and air-conditioned chambers furnished like small hotel rooms (3 x 2.1 m), each with a bed, desk, chair, hand basin, television and video/DVD player, computer, telephone and toilet. Each chamber has three windows, each with interior privacy blinds, looking outside the building (ocean view), into the surrounding laboratory, and into the adjacent chamber. Access to the subjects for the investigators is provided by airlocks through which food and other materials can be passed. Temperature was maintained at 24°c and ventilated with fresh air measured by a solid-state gas sample drying system (Peltier dryer). Oxygen concentration was measured using a paramagnetic oxygen analyser (sable system Inc, PA-IB, Las Vegas USA) and carbon dioxide was measured using an infrared analyser (Sable system Inc, Las Vegas USA). Oxygen and carbon dioxide measurements were corrected to STPD (standard temperature, pressure and dry) from fresh air temperature, water vapour pressure and barometric pressure measurements. Data were collected by means of a data acquisition system, connected to a computer to store and analyse the data. The differential oxygen and carbon dioxide analysers were manually calibrated against fresh air for zero readings and against a span gas and nitrogen each study day. Chamber air was sampled every two minutes. Rates of oxygen consumption and carbon dioxide production were calculated from the measured inflow and outflow according to Schoffelen et al[22] with minor modifications. Specifically measurement noise in the raw data was reduced by smoothing with cubic spline functions; smoothed data accounted for 97.3 ± 2.2% (SD) and 99.96 ± 0.02% of the variance in outlet oxygen and carbon dioxide respectively. Urinary nitrogen was estimated from measured urinary urea. Rates of energy expenditure[23] and fuel utilisation[24] were calculated. Data were expressed per subject (eg $kcal.min^{-1}$). The within subject coefficient of variation for repeated 24 hour measurements in four subjects, of 2.5%, 1.7%,

2.5% for oxygen, carbon dioxide and energy expenditure respectively, compare favourably with similar facilities.

Data analysis

Models

Measures of rates of gas exchange and derived rates of fuel were analysed against measures of body composition as covariates in models of the form:

$y = \alpha + \beta_1 *$fat free mass(kg) $+ \beta_2 * Log_e$(fat mass(kg)).

These models assume that metabolic activity is a linear function of fat free mass[25] with an additional independent effect of body fat mass. Absolute fat mass rather than percentage body fat was used because the fixed absolute nutrient loads delivered across subjects were assumed to be competing with the absolute amounts of endogenous stores represented by the fat mass, and because body fat percentage contains information related to the fat free mass which is already explicit in the models. Fat mass was $\log_e$ (Ln)-transformed under the assumption that any effects would be saturable. It was assumed in these analyses that any effects of gender are a consequence of gender difference in body size and composition[26].

Statistical analysis

Analyses were performed using the JMP package (Version 4.0.1, SAS Institute Inc.). All metabolic data were analysed initially by repeated measures MANOVA (simple or nested repeats) in the models described above.

The period between subject entry and the presentation of breakfast (approximately 30min) was used to estimate fasting (basal) rates of fuel metabolism. In preliminary analyses (simple

repeated measures MANOVA) there were no differences between estimates obtained on the three study days and the fasting data were therefore averaged across the three studies in all subsequent analyses. These averaged data were analysed using the model described above.

In preliminary analyses data obtained from the presentation of breakfast to the exit from the chamber was blocked into two meal related periods of 150 min each and two inter-meal periods. These data were then analysed in a nested repeated measures MANOVA (four repeats in time within study, and three repeats in diet between studies). This analysis found no significant effects of time within study, so all data were then averaged within each study to provide estimates of fuel metabolism during the fed period in subsequent analyses. These averaged data were analysed by repeated measures MANOVA (repeats in diet) using the models described above, with planned contrasts between Control and both high protein diets (protein effect), and between the two high protein diets (GI effect). Since no significant GI effects were detected on any variable (see Table 2) the protein effects are illustrated using multiple linear regression analysis of data averaged across the two high protein studies compared with a similar analysis of the control diet data.

Results

Of the 68 people who responded to study advertisements, 23 were eligible and agreed to participate, and 18 of these completed the 3 study visits. The 5 subjects not completing the 3 visits cited personal reasons and scheduling incompatibilities as their reasons for discontinuing the study. The mean age of the participants was 39.8±SD12.9years, 6 subjects were classified as having a normal BMI, 8 subjects were overweight and 4 were obese. There was also considerable variation in body fat and fat free mass (Table 1).

Table 2 shows the results of the repeated measures MANOVA analysis. Significant overall effects were observed for fat and carbohydrate oxidation in the repeated measures MANOVA with the model for energy expenditure approaching significance (Table 2). As with any repeated measures MANOVA involving covariates, the interpretation of the model must consider the interaction between the dependent variables (in this case dietary type) and the covariates (in this case FFM and LnFat mass). In the post hoc analysis of the planned contrasts for the GI and protein effects are also shown (Table 2), the dietary protein effect was assessed from a comparison of the control diet to both high protein diets, and the GI effect from a comparison of the two high protein diets. The FFM and LnFat effects within the Dietary protein effect were **M**-transformed parameter estimates (interaction terms) relating each covariate to the magnitude of the main effect. No significant GI effects were detected and further analysis of the GI effect was not conducted.

Strong interactions between LnFat and dietary protein on substrate oxidation measures and EE were evident. Interactions between fat free mass values and those for dietary protein and substrate oxidation (but not EE) were significant . To investigate these interactions further, scatterplots, including the least squares regression line are presented in Figure 1 for energy expenditure (Figure 1A and B), fat oxidation (Figure 1C and D). and carbohydrate oxidation (Figure 1E and F) for Ln fat mass and fat free mass respectively. The effects of the high protein diet identified (seen in Table 2) thus reflect the presence of a substantial positive relationship between FFM and fat oxidation. It also reflects a negative relationship between fat mass and fat oxidation and, between fat mass and energy expenditure which are not evident in the high protein-fed state.

Discussion

The expected ubiquitous elevations in fat oxidation and energy expenditure on a high protein diet were not found in this study, possibly because most previous work[4-7,9-12] has been done without substantial variation in body composition. While in our case an effect may have been masked by variation in body composition the consistent failure of previous studies to investigate body composition effects is a significant limitation given clear evidence that fat oxidation is impaired in obesity and in those who were previously obese[17,18]. Our approach is more informative than previous attempts that have divided the study sample into two body composition groups[13] or presented in a format which did not adjust for body composition differences in the study sample[14,15].

Our results present a clear relationship between body composition and the effect of dietary protein on substrate oxidation, suggesting that a high protein intake may prevent the obesity induced decrease in fat oxidation. The higher protein intake resulted in preferential fat utilisation in subjects with higher body fat and a sparing of fat utilisation in those with a lower body fat. A more recent study comparing differences in energy expenditure and substrate oxidation in obese and lean women consuming liquid high protein and control meals found a significant increase in fat oxidation in the obese subjects after the high protein meal and no significant differences in thermogenesis between the protein and control meals when body composition was included as a covariate[16]. Although we did not find similar effects for fat oxidation, our results are consistent with these findings for thermogenesis. Our design differed in that we have investigated the relationship between dietary protein and substrate oxidation and between dietary protein and energy expenditure within the group as a whole. Our results demonstrate both the previously acknowledged negative linear relationship between fat oxidation and body fat on the control diet and the novel finding of a normalising

of this relationship on the high protein meals. Similarly the negative relationship between energy expenditure and body fat was also normalised on the high protein meals.

Lower GI foods have been reported to increase fat oxidation[20], however most research showing a favourable effect of a lower GI meal has been conducted in young healthy populations prior to exercise[27,28]. Meal based studies do not show differences in fat oxidation or energy expenditure when GI is varied in an overweight population[29]. Our results are in agreement with the lack of an acute effect of GI on energy expenditure and substrate utilisation in a meal based approach in a non exercising population.

Limitations to our research include the use of bioelectrical impedance to assess body composition. The use of this method has been questioned particularly in those with abdominal obesity as a greater proportion of total body water (on which the impedance estimate is based) will be in the trunk area which contributes significantly less to total body impedance. In this case the total body impedance will be high compared with the amount of body water[30,31]. Nevertheless, we have validated measurements against dual energy x-ray absoprtiometry in a similar study population[21] (with body fat ranging from 13-51%) and therefore have reasonable confidence in our results. Adaptability to substrate delivery is another consideration. There is evidence that the obese person may adapt more slowly to high fat diets and have a reduced ability to utilise fat[32]. Although fat intake was matched in the control and high protein diets, the results of our study suggest that future research might investigate replacing some of the fat in the meal with protein to determine possible further effects. Finally insulin resistance might be an issue and this was not assessed in our study. Recent research using high fat loads suggests that insulin resistance is positively associated with postprandial fat oxidation

independent of body composition[19]. Further research might consider the impact of insulin resistance on possible effects of high protein diets in altering fat oxidation.

Conclusion

The results of this study suggest that high protein meals may have particularly beneficial effects on energy expenditure and fat oxidation in overweight subjects. This study also demonstrates the need to incorporate body composition including both fat and fat-free components in modelling the response of energy expenditure and substrate utilisation to variations in the macronutrient content of meals. This adjustment may be particularly important in the study populations where body composition is known to vary.

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Appendix 1: Macronutrient composition[1] and menu for the test meals provided in the study.

	Control	High protein low GI	High protein high GI
Energy (kJ)	4064	4034	4240
Protein (g)	34	83	83
Fat (g)	32	33	35
Carbohydrate (g)	139	84	90
Fibre (g)	8	8	5
% protein	14	35	33
% fat	29	30	31
% Carbohydrate	58	35	36
MUFA (%)	50	51	53
PUFA (%)	18	14	10
SFA (%)	32	35	38
Calcium (mg)	788	736	788
Total glycemic load breakfast	50	6	26
GI of breakfast meal	65	49	73
Total glycemic load lunch	39	21	30
GI of lunch meal lunch	65	41	74
MENU	**Breakfast** White decaffeinated coffee Cornflakes, sugar and milk Bread, margarine and jam **Lunch** Cheese sandwich with salad vegetables and pesto Rice pudding White decaffeinated coffee	**Breakfast** White decaffeinated coffee Cheese and tomato omelette bread with margarine and bacon **Lunch** Open sandwich with beef, chutney and salad vegetables Low fat fruit yoghurt White decaffeinated coffee	**Breakfast** White decaffeinated coffee Rice cereal, sugar and milk Spanish omelette and bacon **Lunch** Open sandwich with beef, cheese, salad vegetables and a vinaigrette dressing White decaffeinated coffee

1. The energy values and macronutrient composition of the meals used was calculated using the Foodworks nutrient analysis software program (version 3, 2002; Xyris Software, Brisbane, Australia) using the Australian nutrient database AUSNUT.

Table 1 Baseline characteristics of the study participants

	Male (n=5)	Female (n=13)
Weight (kg)	98.7±9.4 (86.3-111.6)	68.8±9.7 (58.9-77.1)
BMI ($kg.m^{-2}$)	30.2±3.9 (25.0-36.0)	24.9±2.8 (21.4-29.5)
Bodyfat (%)	29.5± 5.1 (16.0-41.9)	32.3±6.1 (24.2-38.1)
Fat free mass (kg)	69.0±4.5 (62.5-74.3)	46.6±4.2 (41.0-55.3)

Values are mean ± standard deviation (range), BMI body mass index

Table 2 Repeated measures analysis of variance of metabolic variables in relation to body composition

y [1]		Diet (postprandial)[3]			Full Model[4]	Dietary Protein Effect[7]				GI Effect
	Basal[2]	Control	HP/HGI	HP/LGI	p	p[5]	Intercept	FFM	LnFat	p[6]
Fat oxidation (kcal.min^{1})	0.52±0.14	0.55±0.2	0.21±0.21	0.34±0.11	0.007	0.004	-4.7**	-.046*	2.23**	0.76
Carbohydrate oxidation (kcal.min^{1})	0.56±0.12	0.83±0.14	1.16±0.18	0.94±0.07	0.003	0.004	3.0*	0.039**	-1.57**	0.96
Energy expenditure (kcal.min^{1})	1.39±0.07	1.67±0.08	1.67±0.07	1.61±0.08	0.070	0.030	-1.5*	-0.009	0.63*	0.27

HP/HGI High protein, high glycemic index, HP/LGI high protein low glycemic index, FFM fat free mass (kg), Lnfat $\log_e$ fat mass (kg). *P<0.05, ** P<0.01

1. Dependent variable in linear models.
2. Basal is the average of the first 30 minutes in the chamber averaged across the 3 study visits.
3. postprandial measures are the average of the postprandial period for each of the 3 study visits
4. Significance of the full repeated measures MANOVA comparing the 3 test diets with body composition as the covariate.
5. The Dietary Protein effect compares the Control diet to both high protein diets in a post-hoc contrast.
6. The GI effect compares the two high protein diets in the postprandial period in a post hoc contrast.
7. The FFM and LnFat effects within the Dietary protein effect are the transformed parameter estimates (interaction terms) relating each covariate to the magnitude of the main effect.

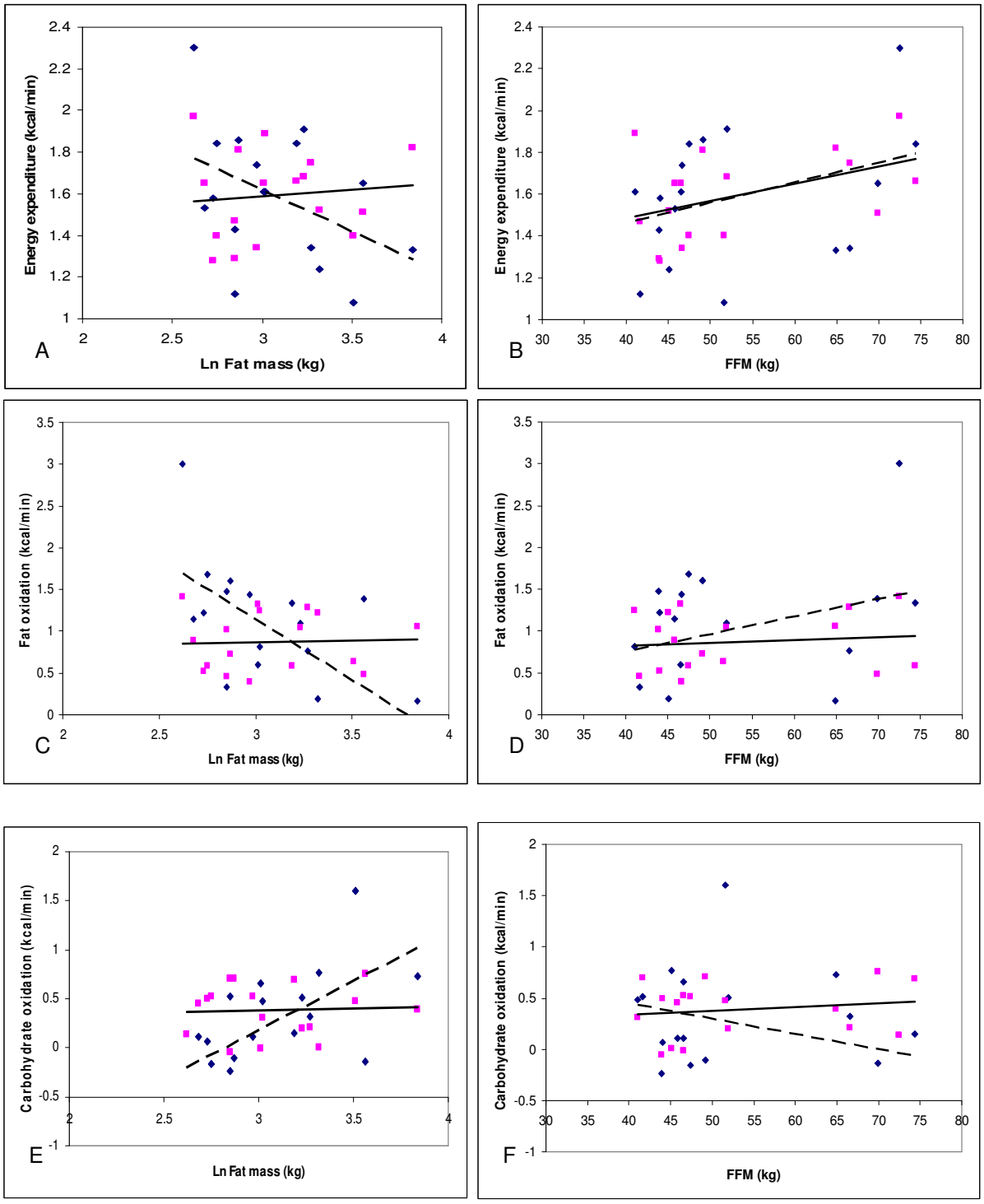

Figure 1 Scatterplots showing the relationship between energy expenditure, fat and carbohydrate oxidation and body composition. ♦ indicate values for subjects on the control meals ■ indicate values for the average of the values for the subjects on the high protein high glycemic index and the high protein low glycemic index meals. Least squares regression slopes

are shown for the control (- - -) and high protein (____) meals. FFM, fat free mass (kg), LnFat, $\log_e$fat mass (kg)