Effect of increasing protein content at the evening meal followed by exercise on overnight nocturnal total energy expenditure, fat and carbohydrate oxidation in healthy young Indian men

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ABSTRACT

Background and Objectives: Indians are more prone to develop diabetes at a younger age, with normal BMI, and this might partly be due to their higher body fat content. Increase in fat mass in the body might be because of the reduction in fat oxidizing capability. Given the fact that Indians consume high carbohydrate diets, effective fat oxidation is likely to be delayed. Simple preventive weight loss strategies like exercise or change in diet regimen are needed to reduce their body fat. This study investigated the effect of exercise with a high protein dinner on overnight thermogenesis and fat oxidation. **Methods and Study Design:** Nine healthy normal subjects aged 18 – 30 years participated in randomised cross over study. They underwent 6 sessions of overnight whole body indirect calorimetry on separate nights with the following experimental conditions: (i) standard (habitual) meal (ii) standard meal with exercise (iii) 20% protein meal (iv) 20% protein meal with exercise (v) 50% protein meal and (vi) 50% protein meal with exercise. Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured overnight, from which energy expenditure, non-protein respiratory quotient (RQ), and fat oxidation were estimated. **Results:** The estimated marginal means of fat oxidation and energy expenditure were significantly different for protocols with exercise compared to those without exercise (p=0.02). There was no acute effect of protein on nocturnal fat or carbohydrate oxidation, with or without exercise. **Conclusions:** Post-dinner exercise increase nocturnal fat oxidation and energy expenditure in young active Indian men.

Key Words: exercise, protein meal, fat oxidation, energy expenditure, indirect calorimetry

INTRODUCTION

Obesity is one of the major risk factors for T2DM (type 2 diabetes mellitus). Among Indians, in addition to obesity, a risk of diabetes,[1,2] has been observed in individuals with low to normal body mass index (BMI), commonly known as the "metabolically obese" phenotype.[3] These are individuals who despite having a normal BMI,[4] might have decreased muscle mass and a higher body fat proportion. It is known that obesity especially increase in fat mass can be due to the reduced ability to oxidize fat.[5] The most common probable cause for increased body fat could be increased energy intake and decreased physical activity. It is also proposed that there could be metabolic inflexibility i.e. inability of an individual to switch from one fuel source to the other, determined by respiratory quotient (RQ).[6,7] Of late, a major focus has been to improve fat oxidation through lifestyle modification, including diet and exercise.[8,9]

Goodpaster et al[10] demonstrated that obese insulin-resistant individuals, when subjected to 16-week exercise regime had increased fat oxidation during exercise and thereby can reduce insulin resistance. In addition, the intensity of exercise, from low to moderate level, augments fat oxidation, but this effect reduces when intensity increases further.[11] In relation to diet, a high protein diet is known to increase postprandial fat oxidation, when compared to carbohydrate and fat based diet.[12] Among various forms of protein, whey protein is suggested to elicit a greater postprandial thermic response than casein or soy.[13] However, no literature is available that has looked at the acute association between macronutrient intake and exercise on nocturnal fat and carbohydrate oxidation among Indian adults of normal BMI. The aim of the present study was to assess the effect of a standard exercise with a high protein dinner on the nocturnal fat oxidation of normal BMI Indians.

MATERIALS AND METHODS

Nine apparently healthy, male volunteers between the age of 18 to 30 years and with normal BMI range (18.5 to 25 kg/m^2) were recruited from, in and around St John's Medical College and Hospital, Bangalore. Subjects were excluded if they were diagnosed with diabetes mellitus, hypertension, metabolic syndrome, with habits of cigarette smoking or alcohol consumption, are on any form of medications like bronchodilators, thyroxin. They were instructed to maintain a regular pattern of diet during the study and were also advised not to perform any strenuous physical activity (physical activity ratio (PAR) >5) on the day of the study. Written informed consent was obtained from all the subjects. The current study was approved by Institutional Ethics Review Committee (IERB/149/2013).

Height and body weight of the subjects were measured using wall-mounted stadiometer and digital weighing scale (Goldtech, India) with minimal clothes and without wearing shoes to the nearest 0.1cm and 0.1 kg respectively. Whole body and regional body composition were estimated using dual X-ray absorptiometry (DXA) scan (Lunar Prodigy Advance PA +301969 (GE Medical Systems, USA), whole body scanner, with software version 12.30.

The experimental design is explained in figure 1. Six protocols (a standard meal, exercise with standard meal, moderate protein meal, moderate protein meal with exercise, high protein meal and high protein meal with exercise) were tested in a randomised longitudinal cross over design (Latin square design). A wash out period of >1 week was maintained between each protocol for a subject. The test duration lasted about 12 hours during the night (5.30 pm till 6 am in the next morning). During the test, oxygen consumption (VO2) and carbon dioxide production (VCO_2) were measured using a whole body indirect calorimeter.13 On arrival, subjects were weighed with minimum clothes after emptying the bladder. At 5.30 pm, they entered the calorimeter chamber and remained there until next morning. Subjects rested inside the calorimetry, watching television or reading. Dinner (see below) was served by 7.45 pm and subjects were instructed to consume the meal within 15 minutes. Following the meal, after a gap of 1 hour, subjects exercised on a treadmill (brisk walk) for a period of 40 minutes, at a speed of 7 km/h, on the specific day of exercise protocol. The speed and inclination of the treadmill were controlled by the experimenter in the adjacent room. The heart rate and oxygen saturation were continuously monitored during the exercise. The subject went to sleep at 10 pm. Gas measurements were continued through the night, until the next morning.

The meal was prepared at the Department of Nutrition kitchen (standard meal (chapatti roll with corn filling and lemon juice) - 10% protein, 20% fat, 70% carbohydrate (CHO); mean energy – 636.4±43.9 kcal or 9.82±1.05 kcal/kg body weight). For introducing extra protein in the meal, whey protein (100% Royal whey protein, National Pro Nutrition, India) was provided. For the high protein protocols, the amount of protein was adjusted according to the percentage desired by reducing the CHO content. Thus, in the 20% protein meal (chapatti roll with corn filling and a glass of whey protein mixed milk), 10% whey protein was added to the diet above, such that the meal contained 20% protein, 20% fat and 60% CHO; with a mean whey protein content of 20.3 g (80 kcal). In the 50% protein meal (chapatti roll with corn filling and a glass of whey protein mixed milk), 40% whey protein was added, such that the meal contained 50% protein, 20% fat and 30% CHO; with a whey protein content of 80.9 g (319 kcal).

The energy content of the meal was determined according to the subject's energy requirement based on their habitual physical activity level (PAL), which was 1.52±0.11. The PAL was calculated by a physical activity questionnaire and the subjects' basal metabolic rate (BMR), using the FAO/WHO/UNU equations. The total daily energy requirement was calculated as the product of PAL x BMR. The energy provided in the dinner was a third of the daily energy requirement.

Whole body indirect calorimetry was performed after dinner and exercise (if present in the protocol) through the night, and in addition to gas exchange, fat and CHO oxidation were also calculated. The indirect calorimeter (13500 mm^3) was furnished with bed, TV, treadmill, table, chair, telephone and sink. The chamber was ventilated with fresh air (186 l/min). VO_2 and VCO_2 (paramagnetic oxygen and infrared carbon dioxide analyzers, Servomex, UK) were measured continuously at 3-minute intervals throughout. Details of calibration of the calorimeter have been previously provided.[14,15] Overnight energy expenditure (EE) and RQ were computed from 11 pm until 6am. Substrate oxidation was calculated from the gas exchange values using stoichiometric equations.[16] EE was estimated by the Weir formula from VO_2 and VCO_2.[17]

All values are expressed as Mean ± SD. The data were normally distributed (as evaluated by a QQ plot). Fat oxidation, CHO oxidation, and EE between protocols were analyzed using a mixed linear model analysis, considering protein and exercise as fixed effects, and order of protocol as a random effect. The interaction of protein levels with exercise was also examined. The predicted marginal means using the least squares method for protein levels and exercise were plotted. The association between % body fat, fat and CHO oxidation was examined using Pearson correlation coefficient. All statistical analyses were conducted using SPSS version 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The level of significance was set at $p<0.05$.

RESULTS

Mean anthropometric parameters of the subjects are shown in Table 1. The mean cumulative overnight fat and CHO oxidation and total EE (corrected for body weight) of the subjects during 6 different protocols are represented in Table 2. The fat oxidation and EE for the protocols with exercise showed an increasing trend compared to non-exercise protocols.

The least square predicted means of mean fat oxidation from the mixed linear model for different protocols are represented in figure 2.a, and shows no significant difference (p=0.92) between the protein levels (0.562 g/kg body weight, 0.555 g/kg, 0.566 g/kg respectively for 10% protein, 20% protein, 50% protein). The fat oxidation was significantly greater for the exercise protocols when compared to non-exercise protocols, irrespective of the protein level (Figure 2b, least square predicted means: 0.535 g/kg, 0.588 g/kg respectively, p=0.02). There was no significant interaction between protein levels and exercise (0.541 g/kg, 0.584 g/kg, 0.523 g/kg, 0.588 g/kg, 0.54 2g/kg, 0.591 g/kg respectively for 10% protein , 10% protein with exercise, 20% protein, 20% protein with exercise, 50% protein, 50% protein with exercise respectively, p=0.93), figure not shown.

Similarly, CHO oxidation measured for 8 hours overnight during 6 different visits were also compared between the protein levels and exercise protocols. While the CHO oxidation was comparable between the three protein levels (Figure 2c, 0.314 g/kg, 0.356 g/kg, 0.356 g/kg respectively for 10% protein, 20% protein, 50% protein, p=0.43) it was higher for protocols with exercise compared to those without exercise (Figure 2d, (0.324 g/kg and 0.360 g/kg, p=0.2). The interaction between protein with exercise were considered, there were no significance between different levels of protocol (0.286 g/kg, 0.341 g/kg, 0.349 g/kg, 0.364 g/kg, 0.337 g/kg, 0.374 g/kg respectively for 10% protein , 10% protein with exercise, 20% protein, 20% protein with exercise, 50% protein, 50% protein with exercise, p=0.85, Figure not shown).

Although predicted means of EE showed an increasing trend with increasing protein levels, it was not statistically significant (Figure 2e, 5.60 kJ/min, 5.69 kJ/min, 5.79 kJ/min respectively for 10% protein, 20% protein, 50% protein, p = 0.32). The EE was significantly greater for exercise when compared to non-exercise protocols (Figure 2f, predicted least square means - 5.4 kJ/min, 5.9 kJ/min respectively, p<0.01). When looked into the interaction of the protein with exercise, it was not significantly different between the levels (p=0.58).

Figure 3a and 3b examine the association of percentage body fat of all the subjects with fat oxidation. Body fat % and fat oxidation were not associated with each other during the standard meal protocol (Figure 3a). When the higher protein diets (20% and 50% protein) or exercise were examined, there was a significant negative correlation between body fat % and fat oxidation (Figure 3b, r=-0.93, p<0.001).

DISCUSSION

The primary aim of this study was to assess the effect of a single bout of exercise after a high protein dinner, on overnight fat and CHO oxidation, and EE in young, healthy adult males under strictly controlled conditions. The main outcome is that the post-dinner exercise significantly increased nocturnal fat oxidation and EE in comparison with non-exercise protocols, while the different composition of protein meals did not show any significant effect, nor was there any interaction with exercise. There was no significant effect on CHO oxidation for the exercise protocols when compared to non-exercise protocols.

Previous studies have reported that there is a shift toward greater fat oxidation in the post-exercise period during recovery[18,19] and is greater after higher intensity exercise bouts.[20] In the present study, we implemented a moderate intensity exercise for the subjects. The results from the earlier studies, support the outcome of the present study, that exercise does increase post-exercise nocturnal fat oxidation and EE. The increase in these parameters might be due to an excitation of sympathetic nervous system outflow during exercise.[20] Beilinski et al[21] demonstrated that in the post-exercise post-meal period, the EE was significantly greater than during the same period on a control day, and suggested that the possible mechanisms for the rise in EE during long-term post-exercise period are hormonal changes or increased body temperature. In the protocol which we followed, the exercise was for one hour after dinner, and the EE was examined in 2 ways: for 2 hours after exercise, as well as throughout the night, to examine the post-exercise consumption of oxygen during the recovery period. There was no significant difference in CHO oxidation during the exercise protocols compared to non-exercise protocols and it is likely to be due to the lesser contribution of the muscle glycogen to the fuel utilization post exercise.[22] In contrast with our findings, Melanson et al[18] found that CHO oxidation increased with increasing intensity of exercise when compared with control days, but this could be because of a different study design, where a meal was given to the subjects after the exercise and post-exercise measurements of substrate oxidation were encompassed with this energy intake.

Another outcome of the present study was that there was no significant effect of introducing a high protein diet before exercise, on overnight fat oxidation. In this protocol, whey protein was to increase the dinner protein content, because animal protein elicits a greater thermogenesis when compared to vegetable protein.[23] Previous studies have found that weight maintenance was more effective for the high protein with a low-fat diet than high CHO low-fat diets because of its greater thermogenic and satiating effect.[24,25] Measurements of substrate utilization in adults after ingestion of different test meals have suggested that cumulative fat oxidation was significantly greater for whey protein meal than soy protein.[13] In contrast to the above findings, in the present study, there was no significant effect observed when considering protein combined with exercise as a fixed factor, though we found an independent significant effect with exercise. The possible mechanism for an enhanced effect of protein is that it could alter intracellular lipolysis by influencing glucagon levels.[26] It has been found in earlier studies that, the postprandial rise in insulin-glucagon ratio will cause changes in the lipids levels in liver and adipose tissue in the body.[27,28] In the current study, post-meal EE was not calculated because the exercise after meal did not allow splitting up of a residual effect of meal from exercise.

We also found that there was no correlation between % body fat and cumulative fat oxidation during standard meal protocol, which replicated habitual dietary patterns. However, when extra protein was provided with or without exercise, there was an association ($r=-0.93$, $p<0.001$). A weight loss study[29] has shown that a high protein diet, provided at the expense of CHO, combined with exercise has additive effects in improving the body composition in women. Studies have also shown that obese insulin-resistant subjects exhibit a lower basal fat oxidation than lean subjects.[30] In contrast, one study[31] found no difference in 24-hour fat oxidation and RQ in obese individuals when compared to lean subjects nor in the 24-hr total fat oxidation response to an acute bout of exercise. Although the subjects who participated in the present study had a normal BMI category, those who had higher body fat mass did have a lower fat oxidation.

One of the limitations of this study was that the exercise was done after the protein meal, so the residual thermogenic effect of protein could not be separated from the exercise-induced effect. We only studied males to avoid the confounding effects of the menstrual cycle in females. Blood samples could not be taken to assess hormonal levels as the subjects were within a chamber.

In the present study, post-dinner exercise had a beneficial effect on nocturnal cumulative fat oxidation and EE with no effect on CHO oxidation. Incorporating higher levels of protein in the dinner did not further increase fat oxidation. The nocturnal fat oxidation was greater for subjects with a lower body fat proportion. This study confirms the importance of post-dinner exercise to increase nocturnal fat oxidation and EE. It is now necessary to employ this protocol on a long-term basis to assess whether it can reduce fat mass.

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AUTHOR DISCLOSURE

The authors declare that there are no conflicts of interest for publishing this study.

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Table 1. Subject characteristics†

Parameter	Mean±SD
Age (years)	22.2 ±3.07
Height (cm)	171.4 ±4.24
Weight(kg)	65.6 ±6.55
BMI (kg/m^2)	22.3 ±1.99
Body fat %	22.3 ±6.55
Fat mass (kg)	14.5 ±5.18
Fat free mass (kg)	49.3 ±2.64

†Data represented as mean±SD, (n=9).

Table 2. Mean fat, CHO oxidation and EE during each protocol

Protocol	RQ	Fat oxidation g/kg	CHO oxidation g/kg	EE kj/min
Standard meal	0.761 ±0.02	0.54 ±0.04	0.286 ±0.12	5.35 ±0.38
Standard meal + exercise	0.761 ±0.02	0.584 ±0.09	0.341 ±0.1	5.85 ±0.34
Moderate protein meal	0.773 ±0.02	0.523 ±0.09	0.349 ±0.09	5.38 ±0.55
Moderate protein + exercise	0.765 ±0.02	0.587 ±0.09	0.364±0.09	5.99 ±0.62
High protein meal	0.769 ±0.02	0.542 ±0.08	0.337 ±0.12	5.58 ±0.37
High protein meal + exercise	0.767 ±0.01	0.59 ±0.07	00.374 ±0.09	6.02 ±0.37

RQ: respiratory quotient; CHO: carbohydrate; EE: energy expenditure.
†Data represented as mean±SD, adjusted for body weight (n=9).

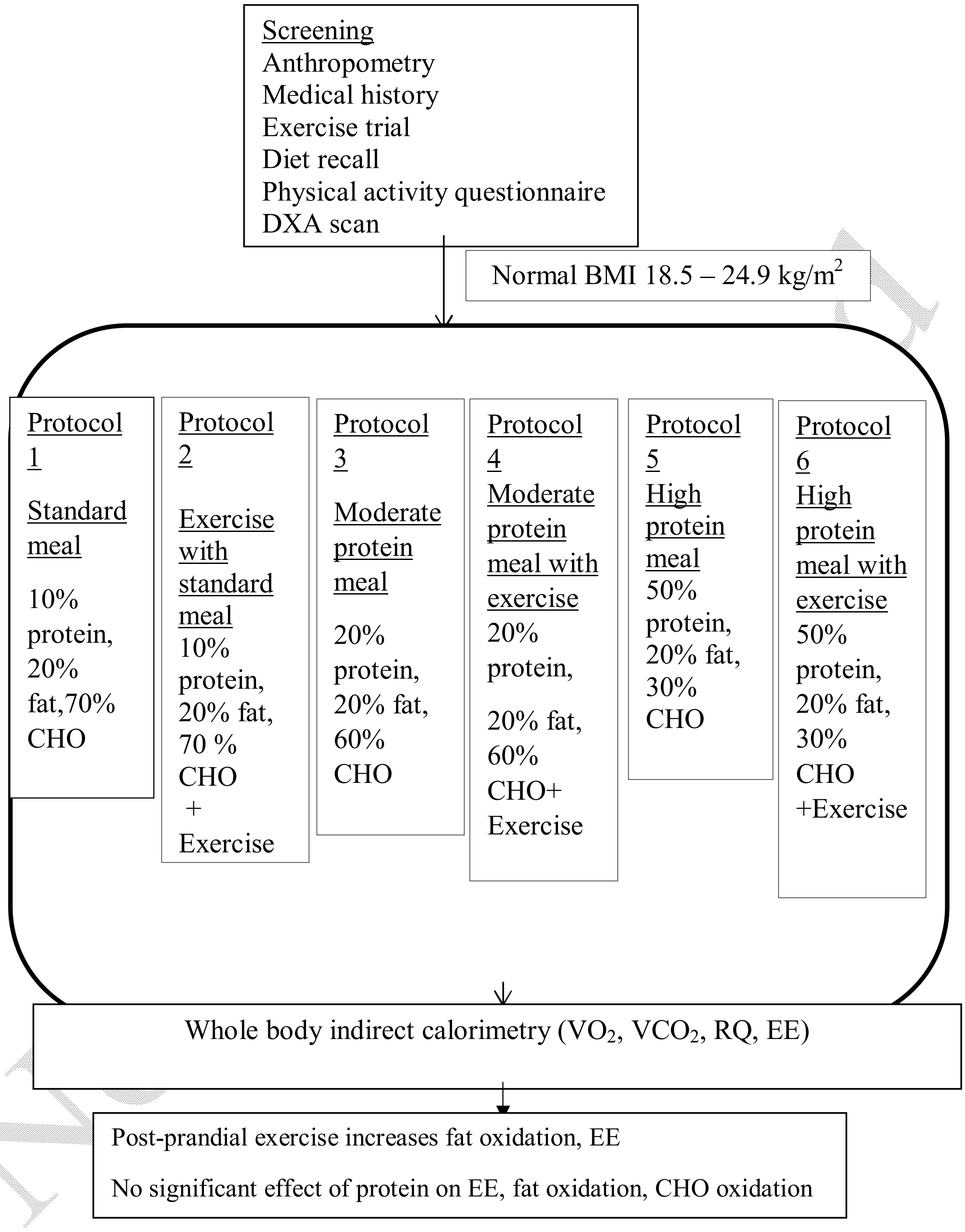

Figure 1. Protocol design followed by the subjects with the main outcome. BMI: body mass index; CHO: carbohydrate; RQ: respiratory quotient; EE: energy expenditure.

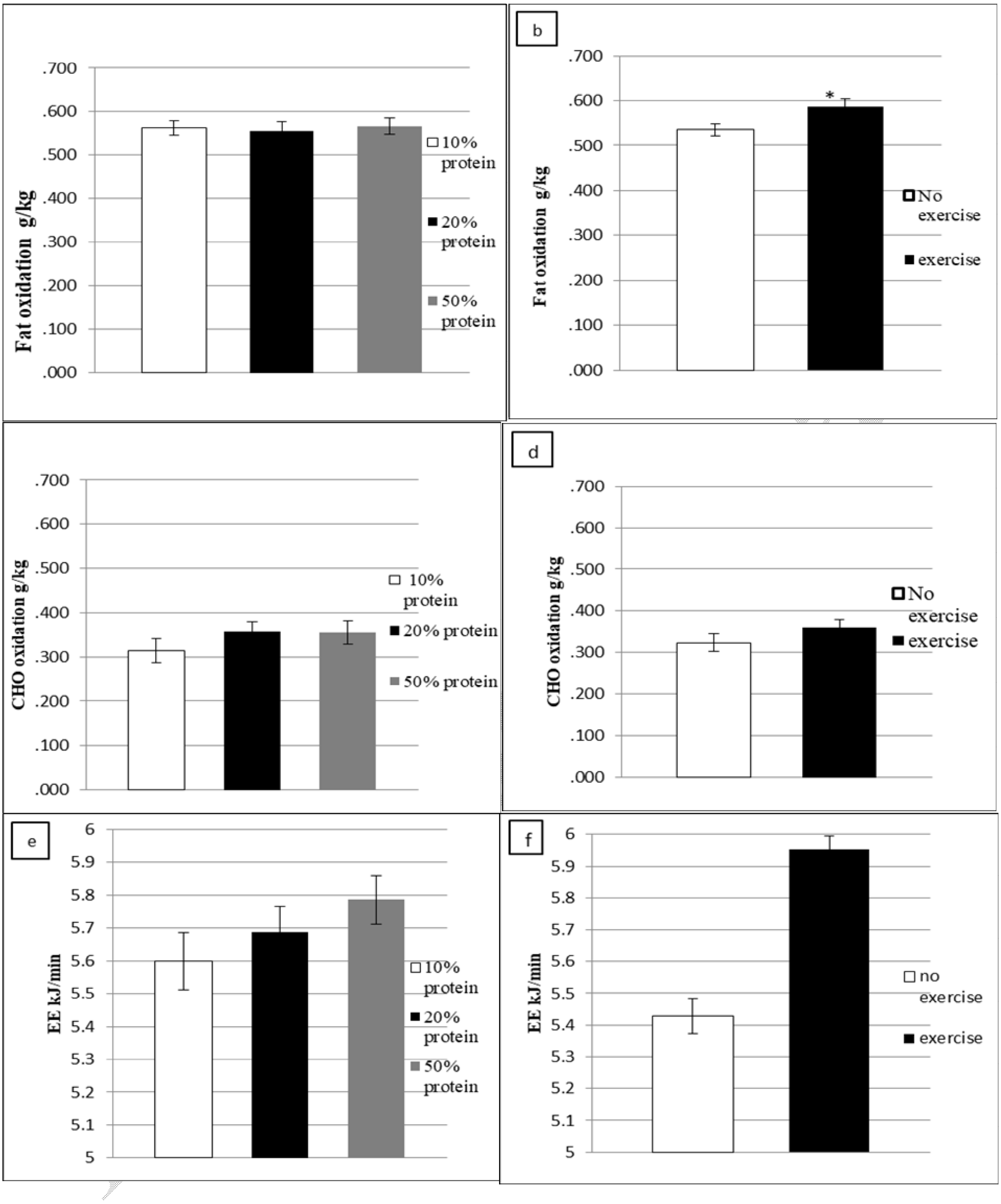

Figure 2. Data represented as estimated marginal mean±SE (Mixed linear model analysis): (a) fat oxidation (protein effect) p=0.92. (b) Exercise effect (p<0.05). (c) CHO oxidation (protein effect) p=0.43 (d) exercise effect p=0.2. (e) EE (protein effect) p=0.32 (f) exercise effect (p<0.01). *Significantly different from that without exercise. CHO: carbohydrate; EE: energy expenditure.

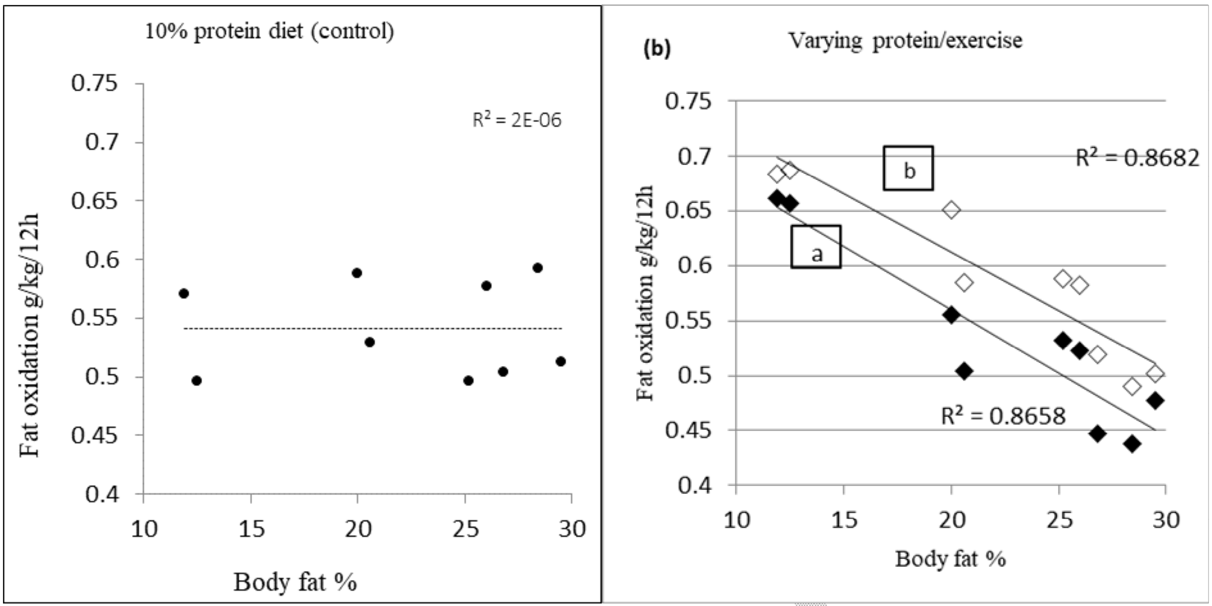

Figure 3. Pearson correlation. Crossover of 6 trials for each subject (mean±SE) n=9; (a) During standard meal protocol. (b) During varying protein with or without exercise. a: mean fat oxidation during 20% and 50% protein for each subject (◆), b: Mean fat oxidation during 20% and 50% protein with exercise (◇).