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Protein intake induced an increase in exercise stimulated fat oxidation during stable body weight☆

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ABSTRACT

Background: Protein-rich weight-loss diets spare fat-free mass at the cost of fat mass. The objective was to examine if there is a change in stimulated fat oxidation related to protein intake during stable body weight.
Methods: Subjects' (BMI 22 ± 2 kg/m^2, age 25 ± 8 years) maximal fat oxidation (Fat_{max}) was assessed during a graded bicycle test, before and after a 3-month dietary-intervention of 2 MJ/day supplements exchanged with 2 MJ/d of habitual energy intake. The parallel design consisted of protein-rich supplements in the protein group and an isocaloric combination of carbohydrate and fat supplements in the control group. Daily protein intake was determined according to 24-h urine nitrogen. Body composition was measured according to a 4-compartment model by a combination of underwater-weighing technique, deuterium-dilution technique and whole-body dual-energy X-ray absorptiometry (DXA).
Results: Subjects were weight stable and did not change their physical activity. The protein group ($n = 12$) increased protein intake (11 ± 14 g, $P<0.05$) and had significantly higher daily protein intake vs. control ($n = 4$) (80 ± 21 vs.59 ± 11 g, $P<0.05$). Fat_{max} increased significantly in the protein group (0.08 ± 0.08 g/min, $P<0.01$). Fat-free mass increased independent of change in body weight ($P<0.01$), and fat mass and fat percentage decreased ($P<0.05$). Change in Fat_{max} was a function of change in protein intake ($r = 0.623$, $P<0.05$), and not of changes in body composition or VO_2max.
Conclusion: Increased stimulated fat oxidation was related to increased protein intake.

1. Introduction

Obesity is a condition in which fat mass (FM) and fat percentage are increased [1] and levels of fat oxidation are suggested to be disturbed [2]. Fat and carbohydrate oxidation are mainly influenced by exercise intensity [3]. With increasing exercise intensity, fat oxidation first increases to its maximal fat oxidation rate (Fat_{max}) from low- to moderate-exercise intensities and then decreases from moderate- to high-exercise intensities [3]. The daily majority of energy demand is at rest or during moderate-exercise intensity. At rest and during moderate-exercise intensity, fat oxidation is the main source of energy production for the body [4]. So, moderate-exercise intensity yields the most grams of fat used for oxidation and could therefore play a role in the maintenance of or reduction in FM. The desired goal for the treatment of and the reduction in development of obesity is to decrease FM while preserving or increasing fat-free mass (FFM). Increased protein intake has shown to result in greater loss of FM and lower loss of FFM during energy restriction, and lower regain of FM and greater regain of FFM during weight regain after weight loss [5–7]. The resulted higher ratio of FFM to FM plays an important role in the maintenance of energy balance [8] and the preservation of metabolic and overall health [9,10]. Since elevated protein intake results in a more favorable body composition during weight loss and weight maintenance thereafter, and since FM is mainly reduced during moderate-exercise intensity, the question remains whether these characteristics hold when subjects are in conditions of energy balance. Therefore, the aim of this study was to investigate whether a change in dietary protein might change stimulated fat oxidation during exercise in subjects with constant body weight over time.

2. Subjects and methods

2.1. Subjects

Subjects were recruited by means of an advertisement in local newspapers and on notice boards at Maastricht University. Subjects who were willing to participate in the study were subsequently screened, by means of a detailed medical history and a physical examination. All subjects were in good health, non-smokers, at most moderate alcohol users, did not use prescription medication, and did not

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fluctuate more than 2 kg in body mass over at least the last 2 months. All subjects gave a written informed consent. The Medical Ethics committee of the University and Academic Hospital of Maastricht approved the study. Twenty-five subjects started in the study, 11 men and 14 women. Eight subjects (5 men and 3 women, and 4 subjects in each group) dropped out due to several reasons, such as personal and an inability to fulfill the schedule with visits to the university. Dropouts were not different from completers in baseline body weight, BMI and body composition, Fat_{max}, physical activity or protein intake. One subject of the control group had excessive protein intake at baseline (216 g at baseline and 89 g during the intervention), and was removed from the analysis. Subject characteristics ($n=16$; 12 in the protein group and 4 in the control group) are given in Table 1.

2.2. Study design

The study had a randomized parallel design and consisted of a dietary intervention period of 3 months. A test day that included measurement of substrate oxidation during a graded bicycle test to exhaustion, measurements of body composition, blood sampling and completing questionnaires took place at baseline and after 3 months of intervention. Subjects were familiarized with the equipment and the procedures before the start of all measurements. Measurements were performed in the morning after an overnight fast. The bicycle-ergometer test started at the same time in the morning to avoid circadian variance. The day before both test days, subjects were asked to refrain from alcohol, refrain from indulgement in strenuous exercise and refrain from eating and drinking after 11:00 PM. Subjects were instructed to maintain their baseline body weight and to maintain their customary level of physical activity during the entire duration of the study.

2.3. Dietary intervention

Subjects were counseled to consume isocaloric diets to sustain body weight by exchanging 2 MJ of their habitual energy intake with 2 MJ supplements. The protein group received protein supplements consisting of milk-proteins to be incorporated within the subjects' habitual diets to increase daily protein intake. These protein supplements were rich in essential micronutrients and were supplied in three sachets daily containing in total of 52 g of milk-protein, dissolved in water to obtain a milk shake, pudding, soup or muesli (Modifast, Novartis Nutrition, Breda, The Netherlands). The control group received isocaloric carbohydrate-fat supplements consisting of a limonade (Karvan Cevitam, Koninklijke de Ruijter, Zeist, The Netherlands) and of olive oil. All subjects were instructed to consume daily at least 200 g of fruit and 300 g of vegetables.

To asses dietary protein intake, subjects completed three 24-h urine collections at baseline and in weeks 6 and 12. Samples were samples were collected with 10 mL H_2SO_4 to prevent nitrogen loss through evaporation, stored frozen at −20 °C, and later analyzed for urinary nitrogen with a nitrogen analyzer (CHN-O-Rapid; Heraeus, Hanau, Germany).

2.4. Anthropometry

To monitor body weight stability, subjects were instructed to measure their body weight daily at home. At the University, body weight was measured 2-weekly using a digital balance (Chyo-MW-150 K, Chyo, Japan; weighing accuracy 0.02 kg) with subjects in underwear, in the fasted state and after voiding their bladder. If body weight fluctuated by > 2 kg from baseline body weight, subjects were instructed to adjust their energy intake to encourage a return to and maintenance of baseline body weight. Height was measured at baseline to the nearest 0.1 cm using a wall-mounted stadiometer (Seca, model 220, Hamburg, Germany). Body mass index (BMI) was calculated by dividing body weight by height squared (kg/m^2).

2.5. Body composition

Body composition was assessed in the fasted state with the 4-compartment model of Lohman [11]. The model was used to calculate percentage fat mass (%FM) from the independently determined whole-body density (Db), total body water (TBW) and total bone mineral content (BMC). Measuring whole-body density, total body water and total bone mineral content separately increases the accuracy of FFM and FM at baseline and after the intervention and is therefore more suitable to determine changes in FFM and FM, especially if subjects sustain their body weight. All measurements were completed within the same

Table 1
Subject characteristics of the 3-month dietary intervention period.

Group	Baseline			3 months		
	Total	Control	Protein	Total	Control	Protein
	$N=16$	$N=4$	$N=12$			
Age (years)	24.6 ± 7.6	24.8 ± 9.2	24.6 ± 7.5			
Body height (cm)	172.7 ± 10.0	172.9 ± 2.1	172.7 ± 11.7			
Body weight (kg)	66.0 ± 8.6	65.5 ± 3.7	66.1 ± 9.9	66.3 ± 8.6	66.1 ± 4.0	66.3 ± 9.8
BMI (kg/m^2)[a]	22.1 ± 1.7	21.9 ± 1.7	22.1 ± 1.8	22.2 ± 1.9	22.1 ± 1.8	22.2 ± 2.0
FFM (kg)[b]	52.1 ± 10.8	51.9 ± 3.4	52.1 ± 12.5*	52.9 ± 11.5	51.1 ± 4.3	53.6 ± 13.2*
FM (kg)[b]	13.7 ± 4.5	13.6 ± 4.6	13.8 ± 4.7*	13.2 ± 5.1	15.0 ± 4.8	12.6 ± 5.3*
FM (%)[b]	21.4 ± 7.7	20.6 ± 6.1	21.6 ± 8.4*	20.5 ± 8.4	22.5 ± 6.4	19.9 ± 9.1*
FFA (μmol/L)[c]	427 ± 121	409 ± 94	433 ± 132	420 ± 169	594 ± 136	391 ± 160
Protein intake (g)[d]	67 ± 17	58 ± 13	70 ± 17*	75 ± 21	59 ± 11	80 ± 21*
Protein intake/body weight (g/kg)[d]	1.0 ± 0.2	0.9 ± 0.2	1.1 ± 0.2*	1.1 ± 0.3	0.9 ± 0.1	1.2 ± 0.2*
Fat_{max} (g/min)	0.43 ± 0.10	0.52 ± 0.12	0.43 ± 0.11*	0.52 ± 0.11	0.55 ± 0.04	0.51 ± 0.11*
VO_2max (mL/min)	2804 ± 855	2549 ± 597	2889 ± 932	2946 ± 862	3004 ± 508	2935 ± 930
VO_2max/FFM (mL/min/kg)	50.6 ± 7.4	46.7 ± 9.5	51.9 ± 6.5	54.1 ± 6.2	57.0 ± 2.3	53.6 ± 6.6
Baecke[e]	9.2 ± 1.4	8.8 ± 1.0	9.4 ± 1.5	9.0 ± 1.0	9.0 ± 0.7	9.1 ± 1.1
Sport[e]	3.5 ± 0.9	3.6 ± 0.7	3.5 ± 0.9	3.5 ± 0.7	3.6 ± 0.7	3.4 ± 0.7
Leisure time[e]	3.5 ± 0.4	3.3 ± 0.4	3.5 ± 0.5	3.4 ± 0.5	3.3 ± 0.4	3.5 ± 0.5
Work[e]	2.3 ± 0.7	2.1 ± 0.2	2.4 ± 0.8	2.1 ± 0.5	2.1 ± 0.2	2.1 ± 0.6

Mean values and standard deviations.
*$P<0.05$, P-value of paired Student's t-test over time, baseline compared to after 3 months.
[a] Body mass index (BMI kg/m^2) was calculated as body weight (kg) divided by height (m) squared.
[b] Body composition of the 4-compartment model of Lohman; %FM = (274.7/Db − 71.4 TBW/BM + 114.6 BMC/BM − 205.03).
[c] Based on 24-h urinary nitrogen content, 3-month value is the average of 1.5 and 3 months.
[d] Plasma concentrations after overnight fasting, $n=15$ at 3 months.
[e] The Baecke total activity index and its activity subscores of sport, leisure time and work.