Nutrition 28 (2012) 566–571

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Spreading intake of a leucine-rich fast protein in energy-restricted overweight rats does not improve protein mass

Solange Adechian Ph.D. [a,b], Didier Rémond Ph.D. [a,b], Claire Gaudichon Ph.D. [c], Corinne Pouyet M.S. [a,b], Dominique Dardevet Ph.D. [a,b], Laurent Mosoni Ph.D. [a,b,*]

[a] INRA, UMR 1019 Nutrition Humaine, Saint Genès Champanelle, France
[b] Université Clermont 1, UFR Médecine, UMR 1019 Nutrition Humaine, Clermont-Ferrand, France
[c] INRA, UMR 0914, UMR INRA/INA-PG/AgroParisTech/Physiologie de la Nutrition et du Comportement Alimentaire, Alimentation Humaine, Centre de Recherche de Paris, Paris, France

ARTICLE INFO

Article history:
Received 14 June 2011
Accepted 29 August 2011

Keywords:
Milk protein fractions
Body composition
Protein metabolism
Wistar rats

ABSTRACT

Objective: Energy restriction decreases fat mass and fat-free mass. Our aim was to prevent the latter using type and timing of protein nutrition as tools.
Methods: Young male Wistar rats were given a high-energy diet for 5 wk and then energy restricted and fed a high-protein diet containing caseins, milk-soluble proteins (MSP), or a casein–MSP mixture ($n = 9$ per group) as the only source of protein for 3 wk. Food intake was spread over 12 h, whereas in a previous experiment rats consumed their daily ration within 2 to 3 h. Weight and food intake were recorded. The body composition was measured by dual-energy x-ray absorptiometry before and after energy restriction. After 3 wk, the hind-limb muscles, the kidney, intestine, liver, and spleen weights, metabolic plasma parameters, and the liver and extensor digitorum longus muscle protein synthesis rates were measured in the postprandial state.
Results: The food intake was similar in all groups. Energy restriction induced a significant decrease in body weight and fat mass ($P < 0.001$) and stopped the slow growth of lean body mass, with no differences between groups. Among all tissues, a significant effect was detected only for the intestine ($P = 0.0012$), with a higher weight in the casein group. Postprandial liver and muscle protein synthesis rates were not different between groups.
Conclusion: When using a high-protein diet spread over 12 h, the nature of the protein intake has no influence on the sparing of lean body mass during energy restriction in young overweight rats.

Introduction

Obesity has reached epidemic proportions: more than 1 billion adults are overweight, and at least 300 million are clinically obese [1]. Obesity is a risk factor for cardiovascular diseases and diabetes and contributes strongly to the global burden of the associated health costs. Obese individuals seeking weight loss often use restrictive diets, which lead to a decrease in adiposity but also to a loss of fat-free mass [2]. This loss of fat-free mass and in particular muscle mass should be prevented because muscle is an emergency store of amino acids that can be used during stresses, allowing an organism to maintain homeostasis. This loss can be limited by including a sufficient amount of protein in the energy-restricted diet [3]. Previously we compared the capacity of caseins (slowly digested milk proteins) with that of milk-soluble proteins (MSP; rapidly digested leucine-rich proteins) to maintain lean body mass in overweight, energy-restricted rats [4]. In the present study, we investigated whether the timing of the intake of these proteins could have an influence on the sparing of lean body mass.

Indeed, in our previous experiment [4], rats consumed their daily ration within 2 to 3 h. We found that, although the regulations of liver and muscle protein metabolisms were not the same, the final nitrogen balance (and thus whole-body protein mass) was not different between groups. In that experiment, postabsorptive muscle protein synthesis rates were higher in the casein-fed group than in the MSP-fed groups [4]. Given the results obtained in test-meal studies in humans [5–7], we postulated that the muscle protein balance (i.e., protein synthesis

This project was supported by French National Research Agency and by the Centre National Interprofessionnel de l'Economie Laitière.
* Corresponding author. Tel.: +334-7362-4827; fax: +334-7362-4755.
E-mail address: laurent.mosoni@clermont.inra.fr (L. Mosoni).

0899-9007/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.nut.2011.08.020

minus proteolysis) was probably lower in the casein-fed group than in the MSP-fed group in the postprandial state and that the reverse occurred in the postabsorptive state, so that the overall 24-h balance was the same. To expand on these recent findings, we postulated that, by spreading protein intake over 12 h, a high postprandial muscle protein balance could be maintained in the MSP-fed group by its high leucine content, a key amino acid for protein synthesis stimulation [8], and by limiting the duration of the postabsorptive period to limit postabsorptive muscle losses. This would be the equivalent of four meals a day in humans, whereas in our previous experiment it was more equivalent to one meal a day.

Thus, in the present experiment, young male Wistar rats were fed ad libitum for 5 wk with a high-energy diet [9] and then energy restricted and fed a high-protein diet containing caseins, MSP, or a casein–MSP mixture for 3 wk. Food intake was spread over 12 h: four equal meals were distributed by an automatic delivery system. We used dual-energy x-ray absorptiometry (DEXA) to assess the changes in lean body mass, and we measured muscle mass and in vivo postprandial protein synthesis rates.

Materials and methods

Animals and diets

This study was performed in accordance with current legislation on animal experimentation in France. Male Wistar rats ($n = 27$; Harlan, Gannat, France) weighing 325.0 ± 1.3 g (mean ± standard error) were housed individually in cages under controlled environmental conditions (temperature 20 ± 1°C, humidity 50 ± 5%) with a 12-h inverse light/dark cycle (light on at 17:00 h) and free access to tap water. On arrival, rats were acclimated to the animal facilities for 5 d and fed ad libitum with commercial laboratory pellets (UAR 04, UAR, Villemoisson sur Orge, France). Then, animals were fed ad libitum a high-fat, high-sucrose semiliquid diet (Table 1) during 5 wk. We showed previously that this diet increases animal fat mass [9]. Rats were then randomly divided into three groups: each group was fed with a restricted amount of a diet containing caseins ($n = 9$), MSP ($n = 9$) or a mixture of casein and MSP ($n = 9$; 50/50 w/w) as the only source of protein (Table 1) for 3 wk. Our aim was to provide restricted rats with 60% of their usual spontaneous energy intake (estimated at 355 kJ/d in a separate experiment) and to make sure that energy-restricted animals did not consume their food in one rapid meal (meal feeding at 05:00, 08:00, 11:00, and 14:00 h, with removal of food at 17:00 h). However, because of the change in their environment (automatic delivery system, change from a semiliquid diet to a dry powder), animals consumed less than expected (overall, only 78% of what was offered to them) and progressively increased their intake during the 3 wk of feeding. Food intake was recorded during the entire experiment by measuring the dry matter intake daily.

Table 1
Diet composition

Ingredient	High fat/high sucrose	Casein	MSP	Mixture
L-Cystine (g/kg diet)	1.2			
Total milk proteins (g/kg diet)	179.0			
Casein (g/kg diet)	—	394.2	—	197.0
MSP (g/kg diet)	—	—	413.0	206.5
Starch (g/kg diet)	68.9	325.0	332.0	329.0
Sucrose (g/kg diet)	423.2	—	—	—
Lactose (g/kg diet)	—	31.1	—	15.3
Lard (g/kg diet)	202.8	—	—	—
Soybean oil (g/kg diet)	27.4	120.5	125.8	123.0
AIN-93M mineral mix (g/kg diet)	35.0	58.3	58.3	58.3
AIN-963M vitamin mix (g/kg diet)	10.0	16.7	16.7	16.7
Cellulose (g/kg diet)	50.0	50.0	50.0	50.0
Choline bitartrate (g/kg diet)	2.5	4.2	4.2	4.2
Protein (% energy)	14	37	37	37
Lipid (% energy)	45	28	28	28
Carbohydrate (% energy)	41	35	35	35
Metabolizable energy (MJ/kg diet)	20.4	17.5	18.0	17.8

MSP, milk-soluble proteins

Measurements of in vivo tissue protein synthesis rates

Tracer injection and tissue collection

After 3 wk of restriction, tissue protein synthesis rates were measured in vivo using the flooding dose method [10]. These measurements were performed in the fed state, 4 to 7 h after the beginning of the dark period. The fed state was confirmed by measuring the dry matter content in the stomach. It was positive and similar in the casein (2.1 ± 0.3 g) and MSP (2.2 ± 0.4 g) groups (means ± SE), but in the mixture group, two rats were not used for protein synthesis measurements because their dry matter content in the stomach was low. It was correct for the other mixture-fed rats (1.7 ± 0.2 g). Twenty minutes before euthanasia, each rat was injected in a lateral tail vein under a light gaseous anesthesia (isoflurane; Baxter, Mauripas, France) with a flooding dose of valine (150 μmol/100 g of body weight) to flood the precursor pools, with 50% of L-[1-^{13}C] valine (99%; Cambridge Isotope Laboratories, Andover, MA, USA). The reliability of valine as tracer has been checked previously [11]. Then, anesthesia was induced by an intraperitoneal injection of pentobarbital sodium (Sanofi, Libourne, France) before euthanasia by exsanguination (abdominal aorta). Blood was rapidly collected in heparinized tubes and centrifuged at 3000 × *g* at +4°C for 10 min. Plasma was collected and kept frozen in liquid nitrogen before storing at −80°C until further analysis. The liver and extensor digitorum longus muscle were excised, quickly chilled on ice to stop the tracer incorporation (the liver was cut into small pieces, rinsed in cold saline [NaCl 9 g/L] solution to remove the blood, and wiped), weighed, frozen in liquid nitrogen 3 to 5 min after exsanguination, and stored at −20°C until analysis. The small intestine was emptied, rinsed with cold trichloroacetic acid (0.12 mol/L) dried, and weighted. The epididymal and renal fat pads were carefully removed and weighed. The spleen, kidneys, and gastrocnemius, tibialis anterior, and soleus muscles of both hind legs were also weighed. The stomach content was removed and dried, and its weight was measured. The liver and muscle free and protein bound valine concentrations were determined as described previously [9].

Calculations

The in vivo fractional synthesis rates (FSR; percentage per day) of tissue proteins were calculated as described previously [9]: $FSR = 100 \times (EP - EN)/(EA \times t)$ where t is the incorporation time (expressed in days) and EP and EA are the ^{13}C enrichments of protein-bound valine and of free valine, respectively, at the end of the incorporation time. The incorporation time was measured for each rat from the time of injection to the time of exsanguination and averaged (20.4 ± 2.4 min, mean ± standard error). EN is an estimation of the natural ^{13}C enrichment of protein-bound valine. It was determined in three rats that were not injected with the flooding dose. EP, EN, and EA were expressed in atom percentages. The absolute synthesis rate was calculated from the product of FSR and the protein content of the tissue and expressed in milligrams or grams per day.

Measurements of body weight and body composition

Body weight was recorded three times a week. Whole-body composition was measured in vivo on days 0 and 16 of the energy-restriction period using DEXA (QDR-4500A, Hologic, Inc., Waltham, MA, USA) after a calibration for small animals. Rats were anesthetized by an intraperitoneal injection of a combination of Vetranquil 0.5% (0.250 mL/500 g of live body weight; Sanofi) and Imalgen 1000 (0.376 mL/500 g of live body weight; Merial, Lyon, France) and scanned on a prone position.

Plasma assays

Plasma insulin was analyzed using a commercial radioimmunoassay kit (LINCO Research, Labodia, France). Other plasma measurements were performed using commercial kits from Horiba ABX (Montpellier, France).

Plasma amino acids were purified by ion-exchange chromatography after protein precipitation: 500 μL of plasma was added to 125 μL of sulfosalicylic acid solution (1 mol/L in ethanol with thioglycolate 0.5 mol/L) that had previously completely evaporated. Norleucine was added as an internal standard. Samples were incubated on ice for 1 h and centrifuged at 3500 × *g* for 1 h at 4°C. An aliquot (250 μL) of the supernatant was combined with 125 μL of 0.1 mol/L lithium acetate buffer, pH 2.2. Amino acid concentrations were determined by ion-exchange chromatography (Bio-Tek Instruments A.R.L., St. Quentin Yvelines, France) using postcolumn derivation with Ninhydrin.

Statistical analysis

Results were analyzed by analysis of variance or repeated measures analysis of variance. If the analysis of variance *F* test was significant, post hoc Tukey tests were performed to compare means (SAS, SAS Institute, Cary, NC, USA). Differences between means were considered statistically at $P < 0.05$. Results are expressed as mean ± standard error.