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ScienceDirect

Journal of Nutritional Biochemistry 26 (2015) 9–15

Journal of Nutritional Biochemistry

RESEARCH ARTICLES

Whey-reduced weight gain is associated with a temporary growth reduction in young mice fed a high-fat diet☆

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Received 3 January 2014; received in revised form 17 July 2014; accepted 23 July 2014

Abstract

Whey protein consumption reportedly alleviates parameters of the metabolic syndrome. Here, we investigated the effects of whey protein isolate (whey) in young mice fed a high-fat diet. We hypothesized that whey as the sole protein source reduced early weight gain associated with retarded growth and decreased concentration of insulin-like growth factor-1. Moreover, we hypothesized that these changes were explained by increased nitrogen loss via elevated urea production and/or increased energy expenditure. Male 5-week-old C57BL/6 mice were fed high-fat diets with the protein source being either whey, casein or a combination of both for 5 weeks. After 1, 3 or 5 weeks, respectively, the mice were subjected to a meal challenge with measurements of blood and urinary urea before and 1 and 3 h after eating a weighed meal of their respective diets. In a subset of mice, energy expenditure was measured by indirect calorimetry during the first week of dietary intervention. Observed exclusively during the first week of intervention, whey significantly reduced body length ($P<.01$) and weight gain ($P<.001$) correlating positively with plasma concentrations of insulin-like growth factor-1. The combination diet displayed intermediate results indicating an interactive effect. Urea production, urea cycle activity, food intake and energy expenditure were unaffected by protein source. In conclusion, whey decreased growth-related parameters exclusively during the first week of dietary intervention. The early effect of whey could not be explained by food intake, energy expenditure, urea production or urea cycle activity but was correlated with plasma levels of insulin-like growth factor-1.

Keywords: Whey; Mice; High-fat diet; Insulin-like growth factor-1; Urea

1. Introduction

Consumption of dairy products has been associated with beneficial effects on obesity [1,2] and the metabolic syndrome [3,4]. Milk proteins, which appear to play a positive role, consist of casein and whey protein (whey). Whey in particular, has been demonstrated to alleviate parameters of the metabolic syndrome in humans [5,6] and in rodent models [7–11], including improvement of glucose metabolism, reduction of weight gain and increased fat loss during energy restriction.

The mechanisms of action by whey protein are still not fully established. Whey, compared to casein, has an insulinotropic effect [12,13] which might be caused by the amino acids themselves and by stimulation of the release of incretin hormones as reviewed by Jakubowicz and Froy [14]. Other studies indicate that whey increases thermogenesis [15] which might happen through increased protein synthesis and activation of mammalian target of rapamycin signaling due to whey's high content of leucine [14]. When studying the mechanisms behind the beneficial effects of whey protein intake, it is important to consider the differences in absorption kinetics between whey and casein. Whey proteins are quickly absorbed and cause a rapid, high but transient postprandial rise in plasma amino acids, while casein precipitates in the stomach causing a slower rise and long-lasting plateau of plasma amino acids [16].

In a previous study, we demonstrated a significantly reduced weight gain by whey in high-fat fed young mice during the initial weeks of the dietary intervention, after which the effect ceased [11]. This finding agreed with some other whey studies in somewhat older rodents [8,10] according to the reported weight curves; however emphasis of those studies was on terminal body weight possibly overlooking an important early effect of whey. As the rapid uptake of amino acids from whey has been shown to lead to an increased total dietary nitrogen loss to urea compared to casein, and thereby a suboptimal utilization of dietary nitrogen [17], we suggested that the whey protein uptake in the mouse study was too rapid to be utilized for growth until a certain adaptation had occurred. Since catabolism

☆ Funding: This work was supported by the Danish Strategic Research Council (Grant No. 2101-07-0094; "Tailored Milk for Human Health") and the Danish Dairy Research Foundation. The animal experiments were carried out in the Rodent Metabolic Phenotyping Center, a core facility of the research program of the UNIK: Food, Fitness & Pharma for Health and Disease (see www.foodfitnesspharma.ku.dk) also supported by the Danish Ministry of Science, Technology and Innovation. The funding bodies have not in any way been involved in study design, data interpretation or writing of the manuscript.
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http://dx.doi.org/10.1016/j.jnutbio.2014.07.009
0955-2863/

of amino acids is an energy-consuming process [18,19] a potential hepatic amino acid overflow would, at least partly, explain the reduced weight gain by whey.

Thus, the objective of the present study was to further investigate the early effect of whey on weight gain in mice fed a high-fat diet. Based on our earlier results [11], we hypothesized that urea production and excretion would increase postprandially in whey-fed mice and that the effect would be most pronounced at the early stage of the intervention. Therefore, we analyzed urea concentrations in plasma and urine as well as marker genes for the urea cycle after a meal challenge with whey, casein or a mix of the two, as protein source. Moreover, growth in mammals is strongly regulated by insulin-like growth factor-1 (IGF-1) [20], and a human study by Hoppe et al. [21] indicates that casein, but not whey, causes increased circulating IGF-1 levels. Hence, we hypothesized that the early reduction in weight gain was explained by a growth reduction induced by decreased plasma levels of IGF-1. In order to determine if the decreased weight gain was explained by increased energy expenditure as suggested by Acheson and coworkers [15], we also investigated the effects of whey on energy expenditure during the first week of dietary intervention.

2. Methods and materials

2.1. Ethics statement

The study design was approved by the National Committee for Animal Experimentation, Ministry of Food, Agriculture and Fisheries, Copenhagen, Denmark (License Number: 2012-15-2934-00256 C1).

2.2. Experimental design

2.2.1. Meal study

Seventy-two male C57BL/6NTac mice (Taconic, Laven, Denmark) were purchased at the age of 3 weeks and housed in groups of six with free access to food and acidified tap water. Temperature was maintained at 21–22°C, the relative humidity was 55%± 10% and the light was automatically switched off from 6 p.m. to 6 a.m. During 2 weeks of acclimatization, all mice were fed a standard rodent diet (Altromin 1319; Brogaarden, Lynge, Denmark). At the age of 5 weeks, the cages were matched by body weight and divided into four experimental groups ("HF casein," "HF casein/whey," "HF whey" and "LF casein") to be fed a high-fat diet with casein, a high-fat diet with both casein and whey protein isolate, a high-fat diet with whey protein isolate, and a low-fat control diet with casein, respectively, as described below. The groups were fed the respective test diets for 1, 3 or 5 weeks followed by a meal challenge and subsequent euthanasia. At each time point, two mice were picked randomly from each cage, i.e., the number of animals per cage changed from six to four to two during the study period. Body weights and food intake were recorded twice weekly, and body composition was analyzed weekly in unanesthetized mice by quantitative magnetic resonance imaging (MRI) using EchoMRI 4-in-1 (Echo Medical Systems, Houston, TX, USA). Feed efficiency was calculated as body weight gain/cage (mg) for the specific period divided by energy intake/cage (kJ) for the same period. Prior to the meal challenge, mice were fasted overnight (10 h) in individual cages. After baseline (0 h) collection of blood and urine, the mice had free access to a known amount of their usual test diet for 1 h which was then removed and weighed. Blood and urine were collected at food removal (1 h) and again 2 h later (3 h). The mice were euthanized by cervical dislocation, body length was measured from the thoracic inlet to the caudal rim of the pelvis and tissues were collected for analyses (see below). Blood at 0, 1 and 3 h was collected in EDTA-coated capillary tubes by tail vein puncture and subsequently centrifuged for plasma. Urine was collected in uncoated microtubes by spontaneous urination during handling.

2.2.2. Energy expenditure study

Thirty-two male C57BL/6NTac mice (Taconic) were purchased at the age of 3 weeks and housed in groups of four with free access to chow (Altromin 1319; Brogaarden) and acidified tap water. At 4 weeks of age, mice were placed in individual habituation cages for 3 days and then transferred to a calorimetry system for baseline energy expenditure measurements by a 16-cage indirect calorimetry system (Phenomaster; TSE Systems, Bad Homburg, Germany) for 4 days (TSE system setup; flow 0.4 L/min and sample interval 20 min). Oxygen consumption rate (VO_2; ml/h/kg lean body mass), respiratory exchange ratio (RER), total activity (beam breaks), and food and water intake were measured simultaneously for each mouse. At 5 weeks of age, the same diets as described for the meal study were implemented in the calorimetry system and the effect of the changed diet on energy expenditure was measured for 1 week (n=8). Body weight was recorded twice weekly. At termination, mice were MRI scanned and anesthetized with a Hypnorm/Dormicum mixture (Hypnorm: 0.315 mg/ml Fentanyl, 10 mg/ml Fluanisone; VetaPharma Ltd, Leeds, UK). Dormicum: 5 mg/ml Midazolam; Roche A/S, Hvidovre, Denmark) injected subcutaneously as a 1:1:2 sterile water solution (0.006 ml/g body weight). Blood was collected by puncture of the periorbital plexus in EDTA-coated tubes and centrifuged for plasma which was stored at −80°C together with collected tissues for future analyses.

Table 1
Detailed diet composition

Group and product code[a]	LF casein (D12450B)	HF casein (D12492)	HF whey/casein (D10082505)	HF whey (D10082504)
Casein, 30 mesh (g)	200	200	100	0
Whey protein isolate[b] (g)	0	0	94.5	189
Energy (kcal/g)	3.7	5.1	5.1	5.1
Protein (kJ%)	18	18	18	18
Carbohydrate (kJ%)	71	20	20	20
Fat (kJ%)	10	62	62	62
L-Cysteine (g)	3	3	3	3
Corn starch (g)	315	0	0	0
Maltodextrin 10 (g)	35	125	125	125
Sucrose	350	68.8	68.8	68.8
Cellulose, BW 200 (g)	50	50	50	50
Soybean oil (g)	25	25	25	25
Lard (g)	20	245	245	245
Mineral Mix S10026 (g)	10	10	10	10
Dicalcium phosphate (g)	13	13	13	13
Calcium carbonate (g)	5.5	5.5	5.5	5.5
Potassium citrate, 1 H_2O (g)	16.5	16.5	16.5	16.5
Vitamin Mix V10001 (g)	10	10	10	10
Choline bitartrate (g)	2	2	2	2

[a] Diets were formulated and produced by Research Diets Inc.
[b] Alacen 895, NZMP.

2.3. Test diets

The HF casein group and the LF casein group were fed the widely used casein-based D12492 and D12450B with 60%, respectively 10 %, of energy from fat (Research Diets Inc., New Brunswick, NJ, USA). The HF whey and HF casein/whey groups received a high-fat diet similar to HF casein, except for the protein source which was replaced 100% or 50 %, respectively, with whey protein isolate (custom made by Research Diets, Table 1). All diets contained the same percent energy from protein (Table 1). The whey protein isolate consisted of undenatured soluble whey proteins processed by ion exchange and ultrafiltration (NZMP, New Zealand). Amino acid profiles of both protein sources are listed in Table S1.

2.4. Plasma and urine analyses

Urea concentrations in plasma and urine samples from the meal study were analyzed by an enzymatic, colorimetric method (Urea Assay Kit, K375-100; BioVision, Milpitas, CA, USA). Intra-assay coefficient of variance was 1.9%, while interassay coefficient of variance was 5.3%. The detection limit was 10 μM according to the manufacturer. IGF-1 was measured in plasma from fasted mice by a commercial ELISA kit (Mouse/Rat IGF-1 Immunoassay, MG100; R&D Systems, Inc., Minneapolis, MN, USA). Intra-assay coefficient of variance was 2.7%, and interassay coefficient of variance was 10.0%. Assay sensitivity was 8.4 pg/ml according to the manufacturer. Both assays were performed according to the manufacturers' instructions.

2.5. RNA isolation, cDNA synthesis and gene expression analyses by quantitative polymerase chain reaction

Immediately after euthanasia, hepatic samples were incubated in RNA*later* RNA Stabilization Reagent (Qiagen, Hilden, Germany) for 24 h and afterward stored at −80°C. The samples were subsequently homogenized in lysis buffer (MagMAX-96 RNA Isolation Kit; Ambion, Carlsbad, CA, USA) using glass beads and the FastPrep instrument (FP120, Bio 101, Thermo Savant; Qbiogene, Strasbourg, France), and total RNA was extracted by the MagMAX Express system (Applied Biosystems, Carlsbad, CA, USA) using the MagMAX-96 RNA Isolation Kit (Ambion), following the supplier's protocol. cDNA was produced from~500 ng total RNA by using High-Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems) according to the manufacturer's instructions. Gene expression of the urea cycle-related enzymes arginase 1 (Arg1, Mm00475988_m1), carbomol-phosphat synthase 1 (Cps1, Mm01256489_m1), and glutamic pyruvic transaminase (Gpt, Mm00805379_g1) was analyzed. These genes were selected, since they represent enzymatic steps at different stages of the urea-cycle; the cytosolic *trans*-amination to form glutamate, which is the NH_3 donor to the urea cycle (Gpt), the initial mitochondrial step, in which carbamoyl phosphate is formed from HCO_3^- and NH_3 (Cps1) and the final step of the cycle where urea is finally