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Diets enriched in whey or casein improve energy balance and prevent morbidity and renal damage in salt-loaded and high-fat-fed spontaneously hypertensive stroke-prone rats☆

Arashdeep Singh[a], Adel Pezeshki[a,1], Rizaldy C. Zapata[a], Nicholas J. Yee[a], Cameron G. Knight[b], Ursula I. Tuor[c], Prasanth K. Chelikani[a,*]

[a]Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary, Alberta, Canada
[b]Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, Alberta, Canada
[c]Department of Physiology and Pharmacology, Cumming School of Medicine, University of Calgary, Alberta, Canada

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Abstract

High-fat diets induce obesity and increase risks of diabetes and cardiovascular and renal disorders. Whey- or casein-enriched diets decrease food intake and weight gain; however, their cardiovascular and renal benefits are unclear. We determined whether whey- and casein-enriched diets improve energy balance and are protective against renal damage and morbidity associated with stroke in an obesogenic and hypertensive experimental setting. We also assessed whether the hypophagic effects of these diets were due to reduced diet preference. In experiment 1, spontaneously hypertensive stroke-prone rats were randomized to (a) control (CON; 14% kcal protein, 33% fat), (b) whey (WHY; 40% protein, 33% fat), (c) casein (CAS; 40% protein, 33% fat) or (d) chow (CHW; 24% protein, 13% fat) for 12 weeks with 1% salt in drinking water for CON, WHY and CAS groups. Our results demonstrated that both WHY and CAS produced short-term hypophagia, moderately increased energy expenditure and decreased respiratory quotient, body weight and lean mass, with effects of WHY being more prolonged. Further, only WHY decreased fat mass and blood pressure. Importantly, both WHY and CAS prevented morbidity associated with stroke and decreased indices of renal inflammation (tumor necrosis factor-α, interleukin-6) and damage (osteopontin, renal lesions). In experiment 2, following four initial conditioning trials, the preference for CON, WHY or CAS diet was determined. Both WHY and CAS decreased food intake during conditioning and decreased preference. In conclusion, diets enriched in whey or casein improved energy balance, increased survival and prevented renal damage in salt-loaded and high-fat-fed spontaneously hypertensive stroke-prone rats.

Keywords: Whey and casein; Stroke-prone rats; High-fat diets; Energy balance; Kidney damage; Body composition

1. Introduction

Dairy food consumption is often associated with multiple benefits on metabolic health. In recent meta-analyses of prospective cohort studies, consumption of dairy products has been associated with a significant reduction in risks for cardiovascular diseases, stroke [1–3], hypertension [4] and metabolic syndrome [5]. The bioactive components of dairy that are receiving increasing attention for their health benefits are the milk proteins-whey and casein. In a recent meta-analysis of 14 randomized control trials involving 626 adults, inclusion of whey protein as a dietary replacement has been associated with a significant decrease in body weight and body fat [6]. Further, in randomized control trials involving obese subjects, dietary whey and casein produced similar reductions in blood pressure [7,8], but whey was found to be more effective than casein in improving insulin sensitivity [9] and in promoting satiety [10]. However, little is known about whether the milk protein fractions produce other health benefits, for example, protection against stroke and chronic kidney disease. Further, understanding of the mechanisms by which whey and casein produce improvements in metabolic outcomes is incomplete.

We [11] and others [12–14] have demonstrated that dietary whey and casein decreased food intake and weight gain and improved diabetic control in obese rodent models. However, these obese rodent models are often normotensive and are not inherently susceptible to hypertension and stroke [15], which limits their application for studying the

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* Corresponding author at: Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary, HS 1871, 3330 Hospital Drive NW, Calgary, Alberta, Canada, T2N 4N1. Tel.: +1 403 210 7652; fax: +1 403 210 6693.

E-mail address: pchelika@ucalgary.ca (P.K. Chelikani).

[1] Current address: Department of Animal Science, Oklahoma State University, Stillwater, OK, 74078, USA.

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cardiovascular, renal and neurological effects of dietary protein. The spontaneously hypertensive stroke-prone (SHRSP) rats are a unique genetic model of human lacunar stroke and were selectively bred from the spontaneously hypertensive rats that have greater propensity to develop stroke [16]. Previous studies have shown that salt loading on a high-fat diet (30% energy from fat) promotes the development of risk factors of metabolic syndrome such as hypertension, chronic kidney disease and stroke in SHRSP rats [17–20]. Recently, it was shown that diets containing 55% whey or casein decreased the incidence of stroke and increased survival in SHRSP rats [21]; however, the underlying mechanisms are unknown. There is some evidence that the gut hormone peptide YY (PYY) mediates the satiety effects of high-protein diets in Sprague–Dawley rats [22] and that a combination of leptin and amylin decreases blood pressure in lean and obese Fisher–Brown Norway rats [23]. However, the effects of dietary macronutrients on energy expenditure, substrate utilization, body composition, glucose tolerance and gut hormones have not yet been characterized in SHRSP rats.

We hypothesized that dietary whey and casein will improve energy balance and protect against morbidity associated with stroke and renal damage in salt-loaded and high-fat-fed SHRSP rats. Our objectives were to investigate the effects of whey and casein on energy balance parameters (food intake, energy expenditure, body weight and composition), glucose and insulin tolerance, gut (PYY, amylin) and metabolic (insulin, leptin) hormones, blood pressure and the onset of morbidity associated with stroke in SHRSP rats. To determine the effects of these diets on renal health, we measured the relative mRNA abundance of markers related to renin–angiotensin system and inflammation, and assessed histopathological lesions in the kidney. Further, to determine whether the hypophagic effects of high whey or casein diets were due to the lower preference for the diets, we conducted a diet preference experiment using naive SHRSP rats.

2. Methods and materials

2.1. Animals, housing and diets

The animal work protocols used in this study were approved by the University of Calgary Animal Care Committee (protocol #AC12-0033). Male SHRSP rats (Strain 324, Charles River, Montreal, QC, Canada) were housed individually in a controlled-temperature (22°C–25°C) and -humidity (21%–24%) environment with a 12-h light–dark cycle (lights off at 1030 h). Daily animal care and maintenance were conducted between 0830 and 1030 h, and food and water were provided *ad libitum* throughout the study. All diets were made in our laboratory and stored at 4°C until used. The ingredients for the diets were purchased from either Dyets, Inc. (Bethlehem, PA, USA) or local grocery stores. Whey protein isolate was donated by Agropur Cooperative (Longueuil, QC, Canada).

2.2. Experiment 1: survival and energy balance study

Thirty-three male SHRSP rats at 4 weeks of age were housed individually in computerized laboratory animal monitoring system (CLAMS) metabolic cages and were adapted on normal chow (PicoLab Rodent Diet 20; LabDiet, St. Louis, MO, USA) for a week. At 5 weeks of age, animals were weight matched (~105–115 g) and randomized to one of four dietary groups (Table 1): (a) control [CON; 14% calories from protein (7% whey protein isolate and 7% casein), 33% fat, 53% carbohydrate, 4.4 kcal/g, n=9], (b) whey (WHY; 40% whey protein isolate, 33% fat, 27% carbohydrate, 4.4 kcal/g, n=8), (c) casein (CAS; 40% casein, 33% fat, 27% carbohydrate, 4.4 kcal/g, n=8) or (d) chow (CHW; 24% protein, 13% fat, 63% carbohydrate, 3.43 kcal/g, n=8) for 12 weeks. Animals on WHY, CAS and CON treatments were fed powdered high-fat diets (33% kcal) and were given 1% salt in drinking water to induce obesity, glucose intolerance, hypertension and stroke. The protein contents of CON (14% kcal) and WHY and CAS (40% kcal) diets were formulated to encompass the range of the daily protein recommendations for humans (10%–35% kcal) [24] and the stroke protection by high-protein (45%–55% kcal) diets in SHRSP rats [21]. The SHRSP rats fed powdered CHW were used as internal controls and had *ad libitum* access to tap water without salt.

2.3. Neurological stroke-related symptom assessment

Animals were monitored daily for the development of stroke-related behavioral deficits and were then given a score of 0 to 4 based on neurological deficit assessment [25]. Other behavioral signs monitored for animals approaching moribund state include lethargy, poor grooming, decreased food consumption, increased water intake, slow movement or immobility, loss of coordination, urinary incontinence, huddled in a kangaroo-type position with hyperirritability, microhemorrhages around the eyes, convulsive rhythmic movement of one forelimb, and extreme and rapid weight loss as described by Smeda [26,27]. These animals have a reduced life span [18], and in the current study, moribund animals were euthanized when they reached their clinical humane end point.

Table 1
Diet composition for experiments 1 and 2

Composition (g kg^{-1})	CON	WHY	CAS
Corn starch	437.9	147.8	147.8
Casein, 80 mesh	77.5	0	445
Whey protein isolate	77.5	445	0
Sucrose	147.8	147.8	147.8
Canola oil	100	100	100
α-Cellulose	50	50	50
AIN-93-MX	35	35	35
AIN-93-VX	10	10	10
L-Cystine	1.8	1.8	1.8
Choline bitartrate	2.5	2.5	2.5
Lard	60	60	60
tBHQ (in oil)	0.0008	0.0008	0.0008
Total amount (g)	1000	1000	1000
Protein (% kcal)	14%	40%	40%
Carbohydrate (% kcal)	53%	27%	27%
Fat (% kcal)	33%	33%	33%
Energy density (kcal/g)[a]	4.40	4.40	4.40
Protein: carbohydrate calories	0.26	1.48	1.48
Protein: fat calories	0.42	1.21	1.21

[a] Energy density is calculated from the calorifc values of protein, fat and carbohydrate at 4, 9 and 4 kcal/g, respectively.

2.4. Food intake, water intake and energy expenditure measurements

Food consumption and energy expenditure were monitored (from 1030 h to 0800 h) using the CLAMS system (Columbus Instruments; Columbus, OH, USA) as described previously [11]. Daily water intake was recorded manually by weighing water bottles. Food spillage was recorded manually and used to correct the daily food intakes before any statistical analysis. As SHRSP animals on chow diet had greater spillage, their hourly food intake was not included in the hourly food intake analyses. The volume of oxygen consumed (VO2, ml/kg body weight/h), carbon dioxide produced (VCO2, ml/kg body weight/h) and respiratory exchange ratio were recorded frequently by indirect calorimetry (CLAMS setup; 2-L/min flow and sample interval 40–48 min). The total energy expenditure was computed following a previously published protocol [11] using the following equation: 3.815×VO2 (L/h)+1.232×VCO2 (L/h), and data were represented as kcal/h/kg lean body mass.

2.5. Body weight and body composition measurements

Body weight was recorded twice a week between 0830 to 1030 h using a conventional weigh scale, and body composition was measured weekly in the unanesthetized rat by a quantitative magnetic resonance method using a Minispec LF-110 NMR Analyzer (Bruker Optics, Milton, ON, Canada).

2.6. Intraperitoneal glucose (IPGTT) and insulin (IPITT) tolerance test

For IPGTT, after overnight fasting (~16 h), an IP injection of 50% dextrose solution at a dose of 2 g/kg body weight was administered at 6 and 10 weeks following the initiation of dietary treatments. For IPITT, after overnight fasting (~16 h), an IP injection of insulin at 1 IU/kg body weight was administered at 8 weeks following initiation of dietary treatments. Blood glucose concentrations were determined from the saphenous vein using a hand-held glucometer (Accu-Chek glucose meter; Roche Diagnostics, QC, Canada) at 0, 30, 60 and 120 min after dextrose or insulin injections.

2.7. Blood pressure measurements

Blood pressure measurements were recorded at 4 and 6 weeks using a tail-cuff sphygmomanometer (Coda System, Kent Scientific Corporation) according to the procedure mentioned previously [28]. Briefly, rats were acclimatized to Bollman restraint cages for 2 days prior to measurement of blood pressure subsequently taken between 0900 and 1300 h. On the day of measurement, rats were restrained and their tails were prewarmed to 34°C for 10 to 15 min. At least five measurements were taken, and the average of the three most stable blood pressure recordings at each time point was considered for statistical analysis.