Dietary Whey and Casein Differentially Affect Energy Balance, Gut Hormones, Glucose Metabolism, and Taste Preference in Diet-Induced Obese Rats1–3

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Abstract

Background: Dietary whey and casein proteins decrease food intake and body weight and improve glycemic control; however, little is known about the underlying mechanisms.

Objective: We determined the effects of dietary whey, casein, and a combination of the 2 on energy balance, hormones, glucose metabolism, and taste preference in rats.

Methods: In Expt. 1, Obesity Prone CD (OP-CD) rats were fed a high-fat control diet (33% fat energy) for 8 wk, and then randomly assigned to 4 isocaloric dietary treatments (n = 12/group): the control treatment (CO; 14% protein energy from egg white), the whey treatment (WH; 26% whey + 14% egg white), the casein treatment (CA; 26% casein + 14% egg white), or the whey plus casein treatment (WHCA; 13% whey + 13% casein + 14% egg white) for 28 d. Measurements included food intake, energy expenditure, body composition, metabolic hormones, glucose tolerance and key tissue markers of glucose and energy metabolism. In Expt. 2, naïve OP-CD rats were randomly assigned to 3 groups (n = 8/group). During an 8 d conditioning period, each group received on alternate days either the CO or WH, CO or CA, or CO or WHCA. Subsequently, preferences for the test diets were assessed on 2 consecutive days with food intake measurements at regular intervals.

Results: In Expt. 1, food intake was decreased by 17–37% for the first 14 d in the WH and CA rats, and by 18–34% only for the first 4 d in the WHCA compared with the CO rats. Fat mass decreased by 21–28% for the WH rats and 17–33% for the CA rats from day 14 onward, but by 30% only on day 28 in WHCA rats, relative to CO rats. Thus, food intake, body weight, and fat mass decreased more rapidly in WH and CA rats than in WHCA rats. Energy expenditure in WH rats decreased for the first 4 d compared with CA and WHCA rats, and for the first 7 d compared with the CO rats. Circulating leptin, glucose-dependent insulinotropic polypeptide, interleukin 6, and glucose concentrations were lower in WH, CA, and WHCA rats than in CO rats. Plasma glucagon-like peptide 1 concentrations were greater in WH than in CA or WHCA rats. The improvements in glucose tolerance were greater in WH than in WHCA rats. The plasma membrane glucose transporter 4 (GLUT4)–to-total GLUT4 ratio in skeletal muscle was greater in CA and WHCA rats than in CO rats; other markers of glucose and energy metabolism in the adipose and cardiac tissues did not differ. In Expt. 2, during 4 conditioning trials, daily food intake was decreased in WH, CA, and WHCA rats by 26–37%, 30–43%, and 23–33%, respectively, compared with CO rats. Preferences for WH and CA rats were 45% and 31% lower, respectively, than those for CO rats, but that for WHCA rats did not differ.

Conclusion: Together, these data demonstrate that in obese rats, whey, casein, and their combination improve energy balance through differential effects on food intake, taste preference, energy expenditure, glucose tolerance, and gut hormone secretion. J Nutr 2015;145:2236–44.

Keywords: food intake, energy expenditure, gut hormones, glucose metabolism, taste preference, whey, casein, body weight, body composition, peripheral tissues

Introduction

The 2013 guidelines for the management of obesity and overweight in adults recommend inclusion of dietary protein at 25% of total calories together with caloric restriction to promote weight loss (1). In a meta-analysis of randomized clinical trials, dairy consumption together with energy restriction...
has been associated with a reduction in body weight, and fat mass particularly, in short-term studies (2). Dairy foods contain multiple components of which whey and casein proteins have been demonstrated to decrease weight gain and adipose reserves, and to improve diabetic control, in rodent models (3–8). However, the underlying mechanisms by which whey and casein promote weight loss and improve diabetic control remain largely unknown.

Whey protein was found to be more satiating and also more effective than casein in decreasing food intake and/or weight gain in rodent models (3, 6–9). However, it is unclear whether the reduction of food intake by the consumption of whey and casein in rodent models is due to satiety, aversion, or other nonspecific malaise. For example, the anorexigenic effects of whey and casein in rats are ascribed to aversive effects in some (10, 11) but not all (5, 12, 13) studies. The reduction in energy intake after consumption of whey protein is often accompanied by increased tissue expression and plasma concentrations of the lower gut anorexigenic hormone glucagon-like peptide 1 (GLP-1)4 in both humans and rat models (7, 14). However, it is unknown whether the differential satiety effects of whey and casein are due to differences in circulating concentrations and/or actions of GLP-1 and other anorexigenic gut peptides.

Apart from producing hypophagia, whey has also been shown to exert thermogenic effects in rats and mice (4, 7–9), as well as in some (15) but not all (16, 17) studies in humans. Furthermore, whey was found to produce a greater stimulation of energy expenditure than casein in humans (15) but not in mice (3, 4). However, most of these studies were conducted under acute settings, and the relative efficacies of whey and casein in modulating energy expenditure in the long term are relatively unknown.

Whey proteins have been demonstrated to improve glucose homeostasis, insulin sensitivity, and lipid metabolism in both rodent models and humans (8, 15, 18, 19); less is known of the underlying metabolic pathways by which improvements in tissue insulin sensitivity and energy metabolism are achieved. Whey was reported to increase the mRNA expression of insulin receptor, insulin receptor substrate-1, and glucose transporter 4 (GLUT4) in adipose tissue of obese mice (4), and protein abundance of skeletal muscle plasma membrane GLUT4 in lean rats (20, 21). However, it is unclear whether whey and casein exert similar or differential effects on the expression of intermediaries of insulin signaling, glucose transport, and energy sensing in peripheral tissues. Therefore, the objectives of this study were to compare the effects of whey, casein, and a combination of the 2 in diet-induced obese rats on energy balance variables (food intake, energy expenditure, body weight, and composition), glucose tolerance, meal-induced changes in plasma concentrations of metabolic hormones (GLP-1, glucose-dependent insulinoctropic polypeptide (GIP), amylin, IL-6, insulin, and leptin), and the protein abundance of key molecules regulating glucose and energy metabolism in adipose tissue, skeletal muscle, and myocardium. We also assessed whether the hypophagic effects of the dietary interventions are due to decreased preference in an independent Expt.

**Methods**

**Rats and housing**

The University of Calgary Animal Care Committee approved the animal work protocols used in this study (no. AC12–0033). Male Obesity Prone CD (OP-CD) rats (Crl: OP-CD, Strain 463, Charles River) were housed individually in a controlled-temperature (23–24°C) environment with a 12 h light-dark cycle (dark at 1100). Food and water were consumed ad libitum throughout the study. Two Expts. were performed. In Expt. 1, the effects of whey, casein, and their combination on energy balance, body composition, glucose tolerance, gut hormones, and key markers of glucose and energy metabolism in peripheral tissues was assessed in obese rats. In Expt. 2, the taste preference of the rats for the test diets was assessed.

**Expt. 1**

**Diets.** Forty-eight OP-CD rats (~155 g, aged 6 wk) were housed in metabolic cages of the Comprehensive Lab Animal Monitoring System (CLAMS; Columbus Instruments). Rats were adapted to the environment with pelleted rat purified diet (PicoLab Rodent Diet 20; LabDiet) for 4 d and then fed a high-fat control diet for 8 wk with fresh food provided on alternate days (Table 1). When the rats reached ~440 g, they were randomly allocated (n = 12/group) to the following 4 isocaloric high-fat treatments (4.4 kcal/g; 33% energy from fat): 1) the control treatment (CO; 14% protein energy from egg white), 2) the whey treatment (WH; 40% protein energy; 26% whey + 14% egg white), 3) the casein treatment (CA; 40% protein energy: 26% casein + 14% egg white), or 4) the whey plus casein treatment (WHCA; 40% protein energy: 13% whey + 13% casein + 14% egg white) for 5 wk (Table 1). Diets were made in house with the use of ingredients from Dyets and Agropur Dairy Cooperative.

**Food intake, energy expenditure, body composition, and glucose tolerance.** Food intake and energy expenditure were monitored daily by the CLAMS system throughout the study (Supplemental Methods). Volumes of oxygen consumed, or VO₂ mL/kg · h⁻¹, and carbon dioxide produced, or VCO₂ mL/kg · h⁻¹, were measured, and respiratory exchange ratio (RER) was calculated as VCO₂/VO₂. The energy expenditure rate was then calculated as calorific value × VO₂, where the calorific value = 3.815 + 1.232 × RER (22), and the data were expressed as kcal/kg lean mass · h⁻¹. The CLAMS was started at 1100 daily and stopped at 0900 the following day. Between 0900 and 1100, general maintenance and husbandry, including calibration of the sensors and filling of the feeders, were carried out. Furthermore, during this maintenance period, body weight was recorded 2 times/wk and body composition was measured weekly with the use of a Minispec LF110 NMR Analyzer (Bruker Corporation). An intraperitoneal glucose tolerance test (IPGTT) was performed on all rats at 1 wk and 4 wk on the dietary treatments as we previously described (23). To minimize the carryover effects of the IPGTT, the behavioral measurements (food intake, energy expenditure, body weight, and body composition) were assessed whether the hypophagic effects of the dietary interventions are due to decreased preference in an independent Expt.

**Surgical procedures, mixed meal infusion, blood sampling and tissue harvesting.** We previously reported that intragastric infusion of a mixed meal leads to an increase in multiple gut hormones in rats (24).

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3 Supplemental Methods, Supplemental Figures 1–4, and Supplemental Tables 1–3 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

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4 Abbreviations used: Akt, protein kinase B; AMPK, adenosine monophosphate–activated protein kinase; CA, casein treatment; CLAMS, Comprehensive Lab Animal Monitoring System; CO, control treatment; COX-IV, cytochrome C oxidase IV; GIP, glucose-dependent insulinoctropic polypeptide; GLP-1, glucagon-like peptide 1; GLUT4, glucose transporter 4; HADH, 3-Hydroxyacyl-CoA dehydrogenase; IPGTT, intraperitoneal glucose tolerance test; OP-CD, Obesity Prone CD; RER, respiratory exchange ratio; SIRT3, sirtuin-3; WH, whey treatment; WHCA, whey plus casein treatment.
In the current study, to avoid the effects of variation in food intake among treatments on gut hormone secretion and glucose concentrations, and minimize the potential confounds of variations in gastric emptying rates from meals with varying composition (25) and their subsequent effect on gut hormone secretion, we determined whether direct and precise intraduodenal infusion of mixed meals containing the treatment diets influences the secretion of gut hormones. After 4 wk of dietary interventions, rats were surgically implanted with duodenal and jugular vein (26) catheters (Supplemental Methods). They then received intraduodenal infusions with the use of a syringe pump (model 22, Harvard Apparatus) of a liquid mixed meal containing protein, carbohydrate, and fat in proportions identical to those in the diets at 0.43 kcal/min for 20 min (total energy load, 8.7 kcal; total volume, 8 mL). Blood samples for hormone assays were collected from the jugular vein at 0, 30, and 60 min after onset of the infusion of mixed meal and blood glucose concentrations were measured at the same time points with the use of a hand-held glucometer (Accu-Chek glucose meter; Roche Diagnostics). Plasma processing and storage was as we described previously (23, 27). The rats were then killed with the use of intraperitoneal sodium pentobarbital at 120 mg/kg (Euthanyl, Bimeda-MTC) and epididymal fat, leg muscle, and heart were collected, rinsed in sterile saline, immediately snap-frozen in liquid nitrogen, and stored at −80°C until further analyses.

Plasma hormone assays. Plasma concentrations of GLP-1, GIP, amylin, IL-6, insulin, and leptin were measured in duplicate with the use of a Milliplex Map rat gut hormone panel (Millipore, Luminex) on a Luminex platform (Bio-Plex 200), following our published procedures (23, 27). The intra-assay coefficients of variation for GLP-1, GIP, amylin, IL-6, insulin, and leptin were 9.66%, 7.75%, 9.54%, 9.81%, 6.61%, and 8.22%, respectively. To test whether the WH, CA, and WHCA improved insulin sensitivity, HOMA-IR was calculated as previously described (27). The following formula was used: HOMA-IR = (G0/I0)/2430, where G0 is the baseline blood glucose concentrations (milligrams per deciliter) and I0 is the fasting blood insulin concentrations (micro-international units per milliliter) at 5 wk.

**Western blot analyses.** Western blot was performed for GLUT4, adenosine monophosphate–activated protein kinase α (AMPKα), cytochrome C oxidase IV (COX-IV), protein kinase B (Akt), 3-hydroxyacyl-CoA dehydrogenase (HADH), and sirtuin-3 (SIRT3) proteins (Supplemental Methods; Supplemental Table 1) in skeletal muscle, adipose, and cardiac tissues following our published procedures (23, 27).

**Expt. 2**
To assess whether the reduction in food intake in the WH, CA, and WHCA rats was due to aversion, under similar conditions as in Expt. 1, preference tests were conducted after a modification of previous procedures (26). Briefly, 24 naïve OP-CD rats (~160 g, aged 6 wk) were housed individually in standard shoebox cages and adapted to the environment for 4 d. Subsequently, rats were weight-matched and randomly assigned to 1 of 3 ad libitum diet groups (n = 8/group) that used the WH, CA, or WHCA. The conditioning (training) period lasted 8 d and the preference tests for 2 d. During the conditioning period, the rats were presented with the choice between the test diet (WH, CA, or WHCA) and the control diet (no dietary intervention). The rats were placed on a 12-h light/dark cycle regimen and had ad libitum access to water and food. The preference tests were conducted during the dark period, and the rats were allowed to choose between the test diet and the control diet in a balanced manner for 2 d. The amount of the test diet and the control diet eaten was measured and recorded. The rats were then killed at 120 mg/kg (Euthanyl, Bimeda-MTC) and the brain, heart, liver, adipose, and gastrocnemius muscle were collected, rinsed in saline, immediately snap-frozen in liquid nitrogen, and stored at −80°C until further analyses.

**TABLE 1** Diet composition for diet-induced obese rats

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control</th>
<th>Whey</th>
<th>Casein</th>
<th>Whey + Casein</th>
</tr>
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<tr>
<td>Corn starch,1 g</td>
<td>437.9</td>
<td>147.8</td>
<td>147.8</td>
<td>147.8</td>
</tr>
<tr>
<td>Egg white (spray-dried),1 g</td>
<td>155</td>
<td>155</td>
<td>155</td>
<td>155</td>
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<tr>
<td>Casein,1 g</td>
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<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
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<td>Whey protein isolate,2 g</td>
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<td>0</td>
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<td>145</td>
</tr>
<tr>
<td>Sucrose, g</td>
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<td>147.8</td>
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<td>AN-93-MX,1 g</td>
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<tr>
<td>AN-93-VX,1 g</td>
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<td>L-Cysteine,1 g</td>
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<tr>
<td>Choline bitartrate,1 g</td>
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<tr>
<td>Lard,1 g</td>
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<td>Tertiary butylhydroquinone (in oil),1 g</td>
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<td>Total amount, g</td>
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<td>1000</td>
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<td>Total fat content, % kcal</td>
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<tr>
<td>Energy density, kcal/g</td>
<td>4.40</td>
<td>4.40</td>
<td>4.40</td>
<td>4.40</td>
</tr>
</tbody>
</table>

1 Diet prepared in-house with the use of ingredients from Dyets.
2 Diet prepared in-house with the use of ingredients from Agropur Dairy Cooperative.

**FIGURE 1** Effects of whey, casein, and a combination of whey and casein or control diets on daily food intake (A), mean EE (B), and body weight (C) in diet-induced obese rats. Values are means ± SEs, n = 12. Labeled means at a time without a common letter differ, P < 0.05. CA, casein treatment; CO, control treatment; EE, energy expenditure; Treat, treatment; WH, whey treatment; WHCA, whey plus casein treatment.
period, on alternate days, each group of rats received the CO or WH, CO or CA, or CO or WHCA in feeding jars (no. 910018, Dyets). The diet composition was identical to Expt. 1 except that 4% Kool-Aid (grape) was added to all diets to mask flavors, and the diets were consumed

FIGURE 2  Effects of whey, casein, and a combination of whey and casein or control diets on body fat mass (A), body fat percentage (B), body lean mass (C), and body lean percentage (D) of diet-induced obese rats. Values are means ± SEMs, n = 12. Labeled means at a time without a common letter differ, \( P < 0.05 \). CA, casein treatment; CO, control treatment; Treat, treatment; WH, whey treatment; WHCA, whey plus casein treatment.

FIGURE 3  Effects of whey, casein, and a combination of whey and casein or control diet on blood glucose concentrations at week 1 (A), blood glucose AUC at week 1 (B), blood glucose concentrations at week 4 (C), and blood glucose AUC at week 4 (D) after an intraperitoneal glucose tolerance test in diet-induced obese rats. Values are means ± SEMs, n = 8–10. Labeled means or means at a time without a common letter differ (\( P < 0.05 \)). CA, casein treatment; CO, control treatment; Treat, treatment; WH, whey treatment; WHCA, whey plus casein treatment.
ad libitum at onset of dark. After conditioning, on 2 consecutive preference test days, each group was simultaneously offered the CO and one of the experimental treatments (i.e., the WH, CA, or WHCA). To avoid potential bias, the position of the feeding jars was alternated during the conditioning and preference testing periods, and food intake was recorded at regular intervals.

Statistical analysis
Repeated measures on food intake, energy expenditure, body composition, body weight, IPGTT, and plasma hormones from Expt. 1 were analyzed with the use of linear mixed models. The univariate linear mixed model used was the following: \( Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ij} \), where \( \mu \) is the population mean, \( \alpha_i \) is the fixed effect of treatment diets \( i \), \( \beta_j \) is the fixed effect of time \( j \), \( (\alpha\beta)_{ij} \) is the interaction of treatment and time, and \( \epsilon_{ij} \) is the residual error. Animal nested in treatment was the random variable on which repeated measures were taken and covariance structures modeled. Based on the smallest values of fit statistics for corrected Akaike’s Information Criterion and Bayesian Information Criterion, the covariance structure of the repeated measurements for each variable was modeled as either heterogeneous compound symmetry, first-order antedependence, autoregressive, heterogeneous autoregressive, or Toeplitz. Discrete data on tissue markers of protein abundance were analyzed by 1-factor ANOVA. Means were separated by Tukey’s post hoc analysis. For Expt. 2, the data from each group of rats were analyzed independently by linear mixed models, similar to Expt. 1, with animal as the random variable. Food intake during conditioning trials was modeled to include fixed effects of dietary treatment, conditioning trial, and treatment \( \times \) trial interactions. For preference tests, the model initially included the fixed effects of treatment, day, and time, and their interactions. Because day was not significant, it was subsequently removed from the overall model, and the mean of the individual food intake on consecutive days was used for modeling the covariance matrices. Planned comparisons of individual treatment means within groups were evaluated by paired \( t \) tests. Data were analyzed with the use of SPSS version 22 and presented as means \( \pm \) SEMs. Statistical significance was declared at \( P < 0.05 \) and trends at \( P < 0.10 \).

Results
Expt. 1
Food intake and energy expenditure. Daily food intake was decreased by 17–37% for \( \sim 14 \) d in the WH and CA rats, and by 18–34% for the first 4 d in the WHCA rats, when compared with the CO rats (Figure 1A; Supplemental Figure 1A–E). Thus, food intake was decreased for a longer period in the WH and CA rats vs. in the WHCA rats. The reduction of food intake in the WH rats occurred during both dark and light periods (Supplemental Figure 2A and B). Compared with the CO rats, the mean daily energy expenditure of the WH rats decreased by 7–9% for 7 d (Figure 1B), with initial reductions in the dark and light periods (Supplemental Figure 2C and D and Supplemental Figure 3A–E). The mean daily energy expenditure and the total AUC for the WH rats on d 4 was \( \sim 7–12\% \) less than for the CA and WHCA rats (Figure 1B; Supplemental Figure 3A and Supplemental Table 2). Although the average daily energy expenditure did not differ between the CO, CA, and WHCA rats, the energy expenditure of the CA and WHCA rats was greater than that of the CO or WH rats at multiple intervals during the dark period on days 4, 7, 11, 14, 21, and 27 (Supplemental Figure 3).

Body weight and composition. Compared with the CO rats, the body weight of the WH rats decreased by 9–10% from day 7, that of the CA rats decreased by 8–12% from day 12, and that of the WHCA rats was transiently decreased by 6–8% from day 12 onward (Figure 1C). Thus, body weights were decreased more rapidly in the WH than CA or WHCA rats. Body composition analysis revealed distinct temporal effects from the treatments (Figure 2A–D). Compared with the CO rats, the fat mass of the WH rats decreased by 21–28% and that of the CA rats decreased by 17–33% from day 14 onward, whereas the fat mass of the WHCA rats was 28–30% lower only on day 28 (Figure 2A). The lean mass of the WH, CA, and WHCA rats was 6–7% less than that of the CO rats on days 14 and 21 (Figure 2C); however, relative lean mass tended to be greater in the CA than in the CO rats on day 28 (\( P = 0.10 \), Figure 2D). Overall, the body weight and body fat of the WH, CA, and WHCA rats decreased, with the reduction occurring within 14 d for the WH and CA rats and by 28 d for the WHCA rats.

IPGTT. Glucose tolerance was similar among groups in the week 1 of the study (Figure 3A and B). However, at 4 wk, compared with the CO rats, blood glucose concentrations and total glucose AUC were decreased by 25–42% and 36% for the WH rats and by 23–26% and 21% for the CA rats, respectively.

**FIGURE 4** Effects of whey, casein, and a combination of whey and casein or control diets on plasma concentrations of GLP-1 (A), GIP (B), leptin (C), IL-6 (D), amylin (E), insulin (F), and glucose (G) in diet-induced obese rats. Values are means \( \pm \) SEMs, \( n = 8–10 \). Labeled means at a time without a common letter differ, \( P < 0.05 \). CA, casein treatment; CO, control treatment; GIP, glucose-dependent insulino-tropic peptide; GLP-1, glucagon-like peptide 1; Treat, treatment; WH, whey treatment; WHCA, whey plus casein treatment.
Glucose excursions did not differ between the WHCA and CO rats (P > 0.10). Blood glucose concentrations and total glucose AUC were lower in the WH rats, but not the CA rats, vs. the WHCA rats.

**Plasma hormones.** Plasma GLP-1 concentrations at 30 min and AUC were greater in the WH than the CO, CA, and WHCA rats (Figure 4A and Supplemental Figure 4A). Plasma GIP concentrations at 30 min were lower in the WH and WHCA rats and tended to be lower (P = 0.09) in the CA rats, and the AUC was lower in the WH, CA, and WHCA rats than in the CO rats (Figure 4B and Supplemental Figure 4B). Plasma leptin concentrations were decreased by 48–64% in the WH rats, by 64–84% in the CA rats, and by 66–83% in the WHCA rats for 60 min after a meal vs. the CO rats (Figure 4C). The leptin AUC was 75%, 81%, and 80% lower in the WH, CA, and WHCA rats than in the CO rats (Supplemental Figure 4C). Plasma IL-6 concentrations were decreased by 60–76% in the WH, 83–86% in the CA, and 67–87% in the WHCA rats for 60 min after a meal vs. in the CO rats (Figure 4D). The IL-6 AUC was 65%, 83%, and 73% lower in the WH, CA, and WHCA rats than in the CO rats (Supplemental Figure 4D). Plasma amylin (Figure 4E) and insulin (Figure 4F) concentrations did not differ between groups. Blood glucose concentrations were decreased by 30–51% in the WH, by 44–47% in the CA, and by 49–55% in the WHCA rats from 30 to 60 min after a meal vs. the CO rats (Figure 4G and Supplemental Figure 4G). Furthermore, HOMA-IR values were lower in the WH (4.1 ± 1.4) and CA (4.2 ± 2.2) rats, and tended to be lower (P = 0.09) in the WHCA rats (6.5 ± 0.5) vs. the CO rats (11.2 ± 1.8).

**Regulatory molecules of glucose and energy metabolism.** Skeletal muscle total GLUT4 protein abundance did not differ between the CO, WH, CA, and WHCA groups (Figure 5A). When compared with the CO rats, the abundance of muscle plasma membrane GLUT4 was greater in the WHCA rats (171%; Figure 5B), and the plasma membrane GLUT4-to-total GLUT4 ratio was significantly higher in the CA and WHCA rats (Figure 5C). Skeletal muscle AMPKα, COX-IV, HADH, Akt, and SIRT3 protein contents did not differ among treatments (Supplemental Table 3). Similarly, in the myocardium and adipose tissue, the protein abundance of GLUT4, AMPKα, COX-IV, HADH, Akt, and SIRT3 did not differ among treatments (Supplemental Table 3).

**Expt. 2**

During conditioning trials, the fixed effect of treatment was significant for the WH, CA, and WHCA groups, with a significant treatment × trial interaction for the WHCA group (Figure 6A–C). Across the 4 conditioning trials, compared with the CO group, food intake was decreased by 26–37% in the WH, by 30–42% in the CA, and 23–33% in the WHCA rats, respectively. During preference testing, the fixed effect of treatment was significant for the WH and CA groups, but not the WHCA rats (Figure 6D–F). The rats showed a significant reduction in preference during the first 6 h of dark period in the WH group (−45% preference; Figure 6D) and CA group (−31% preference; Figure 6E) vs. the CO group; preferences were similar for the CO and WHCA groups (Figure 6F).

**Discussion**

There is substantial evidence that dietary supplementation with whey and casein decrease food intake, weight gain, and adiposity, with improvements in glucose tolerance. However, less is known of the underlying mechanisms of action and whether these benefits are due to altered taste preferences. Therefore, in this study, we investigated the effects of diets enriched in whey and casein or a combination of the 2 on energy balance, glucose metabolism, gut hormones, metabolic markers in peripheral tissues, and taste preference in obese OP-CD rats. Our study revealed several important findings. First, food intake, body weight and body fat were decreased in the WH, CA and WHCA groups. The hypophagic effects were more protracted, and the decrease in weight and body fat occurred more rapidly, in the WH and CA rats than in the WHCA rats. Second, glucose tolerance was improved in the WH and CA, but not the WHCA rats; the improvement was more robust in the WH group. Third, the hypophagic effects of the WH and CA, but not the WHCA, in part may be due to reduced dietary preference, and likely contributed to the
reduction in weight gain and adiposity. Fourth, energy expenditure was differentially affected in the WH and CA groups. The reduction in food intake in the WH rats likely contributed to the reduction of expenditure, whereas the hypophagic effects in the CA and WHCA groups were dissociated from alterations in energy expenditure. Fifth, circulating concentrations of GIP, leptin, IL-6, and glucose were decreased after a meal in the WH, CA, and WHCA rats vs. the CO rats; however, plasma GLP-1 concentrations were greater in the WH rats at 30 min after a meal than in the other groups. Sixth, the ratio of plasma membrane GLUT4 to total GLUT4 content in skeletal muscle was greater in the CA and WHCA groups, which is suggestive of increased GLUT4 translocation to plasma membrane for enhanced glucose uptake by muscle. Thus, diets enriched in whey, casein, and their combination exerted differential effects on energy balance and glucose metabolism. Together, these data demonstrate that protein quality is a key determinant of the reduction in food intake and body weight and improvements in glucose metabolism with high protein diets.

The hypophagic effects observed in the WH, CA, and WHCA groups were in general consistent with most (6–9), but not all (28), studies that have evaluated the effects of high whey or casein in high-fat–fed rat and mouse models. The decreased food intake in the WH rats likely contributed to the reduction of expenditure, whereas the hypophagic effects in the CA and WHCA groups were dissociated from alterations in energy expenditure. Fifth, circulating concentrations of GIP, leptin, IL-6, and glucose were decreased after a meal in the WH, CA, and WHCA rats vs. the CO rats; however, plasma GLP-1 concentrations were greater in the WH rats at 30 min after a meal than in the other groups. Sixth, the ratio of plasma membrane GLUT4 to total GLUT4 content in skeletal muscle was greater in the CA and WHCA groups, which is suggestive of increased GLUT4 translocation to plasma membrane for enhanced glucose uptake by muscle. Thus, diets enriched in whey, casein, and their combination exerted differential effects on energy balance and glucose metabolism. Together, these data demonstrate that protein quality is a key determinant of the reduction in food intake and body weight and improvements in glucose metabolism with high protein diets.

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the hypophagia and reduction in taste preference in part may contribute to the decreased adiposity in the WH and CA groups. In contrast, the delayed reduction in body weight and fat in the WHCA group at day 28 is likely due to the intermittent increase in hourly energy expenditure during the dark period in this group. Thus, temporal differences in body composition between isonitrogenous treatments, together with differences in food intake, taste preference, and energy expenditure, suggest that these effects of the high protein treatments are not due to protein content per se but to the quality or source of the protein. A potential caveat is that the WHCA controls for total protein content but is not matched to the amounts of whey or casein in other treatments. It is likely that the lower dietary content of the WH and CA contributed to the lower effectiveness of the WHCA in decreasing body fat; however, interactions between other combinations of whey and casein on energy balance and body composition remain to be characterized.

In the present study, glucose tolerance did not differ between groups in week 1 of the study, when body weights were similar among treatments. However, glucose tolerance improved in the WH and CA rats at 4 wk, coincident with a decrease in body weight, body fat, and plasma leptin concentrations. These results suggest that the improvement in glucose clearance in the WH and CA groups is likely due to reductions in weight, adipose mass, and plasma leptin concentrations. Importantly, compared with the WHCA group, glucose tolerance improved in the WH group, but not in the CA group, which is suggestive of differential effects of dietary whey and casein (or protein quality) on glucose homeostasis. After infusions of test meals, plasma GLP-1 concentrations were increased with the WH group, but not the CA and WHCA groups, whereas GIP concentrations were decreased by the WH, CA, and WHCA. The greater GLP-1 concentrations in the WH rats likely contributed to the pronounced improvements in glucose clearance and HOMA-IR. Furthermore, in our study, plasma concentrations of the proinflammatory cytokine IL-6 were dramatically decreased in the WH, CA, and WHCA rats, which is suggestive of a reduced inflammatory state and insulin resistance in rats fed high-protein diets. In agreement with our results, whey or casein has been reported to improve insulin sensitivity and decrease proinflammatory signals in rodent models (6, 31, 32).

Previous studies have demonstrated that skeletal muscle and adipose tissue account for nearly 81% of total glucose metabolized (33), with lipotoxicity of the muscle and liver playing a key role in the pathogenesis of insulin resistance (34). To gain insights into the mechanisms by which dietary whey and casein influence energy metabolism in peripheral tissues, we focused on key markers of glucose metabolism and nutrient sensing in the muscle, adipose, and cardiac tissues. We found that the abundance of skeletal muscle plasma membrane GLUT4 protein, relative to total GLUT4, was increased in the CA and WHCA groups; however, we were unable to detect such changes in the WH rats. Furthermore, adipose and cardiac GLUT4 protein abundance did not differ between treatments. These data suggest that the CA and WHCA improve glucose tolerance in part through a GLUT4-dependent mechanism in skeletal muscle. It is unlikely that these GLUT4 alterations are a consequence of body weight changes in our study, because at study termination all protein treatments produced a similar consequence of body weight changes in our study, because at

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**References**


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