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Long-Term Intake of a High-Protein Diet Affects Body Phenotype, Metabolism, and Plasma Hormones in Mice

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Abstract

Background: High-protein diets (HPDs) recently have been used to obtain body weight and fat mass loss and expand muscle mass. Several studies have documented that HPDs reduce appetite and food intake.

Objective: Our goal was to determine the long-term effects of an HPD on body weight, energy intake and expenditure, and metabolic hormones.

Methods: Male C57BL/6 mice (8 wk old) were fed either an HPD (60% of energy as protein) or a control diet (CD; 20% of energy as protein) for 12 wk. Body composition and food intakes were determined, and plasma hormone concentrations were measured in mice after being fed and after overnight feed deprivation at several time points.

Results: HPD mice had significantly lower body weight (in means ± SEMs; 25.73 ± 1.49 compared with 32.5 ± 1.31 g; $P = 0.003$) and fat mass (9.55% ± 1.24% compared with 15.78% ± 2.07%; $P = 0.05$) during the first 6 wk compared with CD mice, and higher lean mass throughout the study starting at week 2 (85.45% ± 2.25% compared with 75.29% ± 1.90%; $P = 0.0001$). Energy intake, total energy expenditure, and respiratory quotient were significantly lower in HPD compared with CD mice as shown by cumulative energy intake and eating rate. Water vapor was significantly higher in HPD mice during both dark and light phases. In HPD mice, concentrations of leptin [feed-deprived: 41.31 ± 11.60 compared with 3041 ± 683 pg/mL ($P = 0.0004$); postprandial: 112.5 ± 102.0 compared with 8273 ± 1415 pg/mL ($P < 0.0001$)] and glucagon-like peptide 1 (GLP-1) [feed-deprived: 5.664 ± 1.44 compared with 21.31 ± 1.26 pg/mL ($P = <0.0001$); postprandial: 6.54 ± 2.13 compared with 50.62 ± 11.93 pg/mL ($P = 0.0037$)] were significantly lower, whereas postprandial glucagon concentrations were higher than in CD-fed mice.

Conclusions: In male mice, the 12-wk HPD resulted in short-term body weight and fat mass loss, but throughout the study preserved body lean mass and significantly reduced energy intake and expenditure as well as leptin and GLP-1 concentrations while elevating postprandial glucagon concentrations. This study suggests that long-term use of HPDs may be an effective strategy to decrease energy intake and expenditure and to maintain body lean mass. *J Nutr* doi: https://doi.org/10.3945/jn.117.257873

Keywords: appetite and energy intake, high-protein diet, metabolic hormones, metabolism and energy expenditure, respirometry and calorimetry

Introduction

Currently, diets with elevated protein content are very popular strategies for the treatment of obesity disorders (1) and to increase muscle mass. Several studies (2–6) have shown that high-protein diets (HPDs) used in overweight or obese adults result in loss of body weight and fat mass and preserve lean

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Supplemental Table 1 and Supplemental Figures 1–4 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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Abbreviations used: CD, control diet; GLP-1, glucagon-like peptide 1; HPD, high-protein diet; RQ, respiratory quotient; TEE, total energy expenditure; VH_2O, water vapor.

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mass. Higher protein dietary intake has been shown to reduce appetite and food intake (4, 7) without inducing conditioned food aversion (8). In rodents, HPDs were confirmed to induce satiety and subsequently reduce energy intake and body weight (9–11). Some authors (12–14), with the use of single high-protein meal intake experiments, reported an alteration of either anorexigenic or orexigenic hormones. Published studies (15, 16) have shown that the beneficial effects of HPDs on appetite and body weight in overweight and obese patients are only temporary and that patients lost body weight initially but regained it after ~6 mo (6). Inadequate nutrition counseling, poor dietary compliance, or both have been suggested as potential causes in several studies (2, 17–19). Animal experiments in rodents treated with an HPD have documented similar decreases in appetite that lasted only for a limited period of time (8–11, 20–22) and ruled out scarce dietary compliance and poor diet quality as potential causes. In the literature, there is a lack of studies on the effects of long-term HPD regimens on energy intake and body weight and only 2 studies extended the experimental observations to a period of 3 (11) or 8 (23) wk.

HPD-induced effects on body composition and appetite were proposed to be mediated by mechanisms regulating food intake and energy expenditure. Mammals derive energy from the oxidation of ingested macronutrients to maintain their body homeostasis (24). Previous studies have shown that mice fed a Western high-fat diet showed an increase in energy absorption and expenditure during the initial 7 d (25), but then their body weight increased progressively throughout the study, even though their energy balance normalized. In acute room calorimetry studies, subjects who consumed high-protein meals showed, in comparison to subjects who consumed high-fat or high-carbohydrate meals (26–30), more elevated energy expenditure and oxygen consumption that were attributed to the digestion of proteins (28). The decreased energy intake in HPD-fed subjects cannot explain the short-term decrease in body weight and fat mass; thus, long-term effects induced by HPDs could be due to an adaptive change in metabolic rate and energy expenditure. A study by Kim et al. (31) showed that a 12-wk HPD (47.9% protein) treatment did not induce significant changes in body weight, energy expenditure, and physical activity. In the study by Schwarz et al. (32), a 50% high-protein intake significantly reduced the effect of high-fat-diet–induced body weight gain and adipose tissue mass and reduced hepatic lipid accumulation compared with mice fed a normal-protein diet. This may potentially lead to the development of more effective dietary interventions to prevent or treat patients with obesity disorders or metabolic syndrome. Therefore, our current study analyzes in C57BL/6 mice the effects of a 12-wk treatment with an isocaloric HPD in comparison to a control diet (CD) on body weight and mass composition, food and water intakes, feeding and drinking behavior, energy expenditure, respirometry, physical activity, and concentrations of a panel of plasma metabolic hormones such as active ghrelin, glucagon-like peptide 1 (GLP-1), leptin, glucagon, insulin, and peptide YY (PYY) in mice after being fed or after overnight feed deprivation. The outcome results of the current study provide an important overview of the effects of long-term use of an isocaloric HPD in normal mice. Understanding the mechanisms by which HPDs affect body composition, energy intake and expenditure, and metabolic hormones is important and might potentially lead to the development of more effective dietary interventions to prevent or treat obesity disorders.

Methods

Animals and diets. To investigate the long-term effects of an HPD on body phenotype, calorimetric variables, and metabolic hormones, age-matched wild-type C57BL/6 mice were individually housed under controlled light-cycle illumination (0600–1800) and temperature (21–23°C) conditions. Food and water were provided ad libitum during the duration of the experiment unless otherwise specified. The mice were then separated into 2 different diet groups (**Supplemental Table 1**). The diets were purchased from Research Diets, and the diet food was stored sealed at 4°C until used. The experimental study protocol was approved by the Institutional Animal Care and Use Committee, of the Veterans Affairs (VA) Greater Los Angeles Healthcare System.

Experimental design. Mice at 10–12 wk of age were divided into 2 different groups, single-housed in standard cages, and fed ad libitum either an HPD (60% of energy as protein; $n = 8$) or a CD (20% of energy as protein; $n = 10$). Body weights were measured at baseline and at weekly intervals throughout the 12-wk study period. Food and water consumption were monitored weekly. Body fat and lean mass were assessed biweekly. Body weight, fat mass, and lean mass values were expressed as percentages of change from the initial measurements performed at baseline. At the end of the study period, all of the mice were single-housed in metabolic cages (Promethion; Sable Systems International) for a period of 5 d for habituation, followed by 3 d of monitoring feeding behavior, water intake, energy expenditure, and physical activity.

Body weight and composition analysis. Murine body mass composition was assessed weekly and expressed as a percentage of the total body net weight by using a quantitative NMR analysis system (EchoMRI-700 4 in 1 composition analyzer; Echo Medical Systems). Mice were conscious and lightly restrained during the scan (~2 min).

Determination of food intake behavior. Analysis of food intake was performed by using the BioDAQ Food Intake Monitoring System for mice (BioDAQ; Research Diets, Inc.) as previously described (33).

Assessment of indirect calorimetry by using the Promethion metabolic system. Indirect calorimetry data were recorded in the studied mice by using a Promethion Metabolic Cage System (Sable Systems) as described previously (34). Mice were acclimated for 5 d in metabolic cages before recording calorimetric variables. Nonrestricted, ad libitum access to the food hopper and water were allowed throughout the study. Mice were also feed-deprived beginning at 1800 for a 24-h period to determine calorimetric variables during feed-deprived conditions. Respiratory gases including water vapor were measured with an integrated fuel cell oxygen analyzer, spectrophotometric carbon dioxide analyzer, and capacitive water vapor partial pressure analyzer (35). Respiratory quotient (RQ) was calculated as the ratio of carbon dioxide production over oxygen consumption. Energy expenditure was calculated by using the Weir equation: kcal/h = 60 × [0.003941 × oxygen consumption (VO_2) + 0.001106 × carbon dioxide production (VCO_2)]. Water intake was measured by using the automated Promethion Metabolic Cage System, which monitors in real time the water hopper to continuously measure the water weight (expressed in g) to calculate water intake and water bouts for a complete characterization of the drinking activity. "Mean water intake" was defined as the total water intake measured during a period of time divided by the number of bouts. Water intake was measured in real time through a weight sensor with a 3-mg resolution that was attached to a water bottle. Total water intake was calculated as the total grams of water consumed during either the dark or light phase. Bouts were defined as the number of times the mouse consumed water. Mean water intake was calculated as the total water intake divided by the number of bouts and the minutes that each mouse spent drinking.

Plasma hormone panel. Blood plasma samples were obtained at the end of the 12-wk study period from each mouse in feed-deprived conditions (feed-deprived: 1800–0900) and in postprandial conditions (overnight feed-deprived: 1800–0900) followed by 1-h postfeeding

(0900–1000). Plasma hormone samples were measured as previously described (33). All of the samples were processed in 1 batch and read via a Luminex 100 reader (Luminex).

Data analysis. A multiple-comparison *t* test was used to evaluate the significance of body weight and body mass composition data between the 2 different diet study groups throughout the study. We used a Sidak post-test in our comparison tests. Cumulative food intake and all BioDAQ variables were calculated for significance by using a 2-factor ANOVA with a Sidak post-test for multiple comparisons. To evaluate the metabolic behavior variables a 2-factor ANOVA was used to compare the dark or light phase mean averages between each murine diet group. The factors included in the 2-factor ANOVA analysis were RQ, VO_2, VCO_2, water vapor production (VH_2O), total energy expenditure (TEE), total activity index, coarse activity index, fine activity index, and mean locomotion speed and time of day (dark and light phase). The metabolic hormone variables for HPD- compared with CD-fed mice in feed-deprived and postprandial conditions were analyzed by using an unpaired *t* test. All analyses and graphs were conducted and created by using GraphPad Prism 6 software.

Results

Effects of a long-term HPD treatment on body weight and fat and lean mass. HPD-fed mice, in comparison to CD-fed mice, lost a significant amount of body weight from week 2 through week 6 [at week 2: 25.20 ± 0.36 compared with 30.23 ± 1.04 g (P = 0.05); at week 4: 25.87 ± 1.37 compared with 31.79 ± 1.22 g (P = 0.01); and at week 6: 25.73 ± 1.49 compared with 32.5 ± 1.31 g (P = 0.003)]; however, from week 8 to week 12 the HPD-fed mice regained weight and their body weight values were not significantly different from those of the CD-fed mice (**Figure 1A**). Body fat mass analysis showed significant differences between the 2 groups as shown in Figure 1B: HPD-fed mice had lower percentages of body fat mass at week 4 (8.46% ± 1.27% compared with 14.95% ± 1.84%; P = 0.04) and at week 6 (9.55% ± 1.24% compared with 15.78% ± 2.07%; P = 0.05). However, from week 8 to week 12 there was no significant difference in body fat mass between HPD- and CD-fed mice (Figure 1B). In the HPD-fed mice, the body lean mass percentages were significantly higher than in the CD-fed group throughout the 12-wk study period beginning at week 2 (88.30% ± 0.92% compared with 81.59% ± 1.68%; P = 0.02), at week 4 (87.08% ± 1.30% compared with 80.95% ± 1.74%; P = 0.04), at week 6 (85.36% ± 1.16% compared with 79.31% ± 1.83%; P = 0.05), at week 10 (83.52% ± 1.72% compared with 77.01% ± 1.63%; P = 0.03), and at week 12 (85.45% ± 2.25% compared with 75.29% ± 1.90%; P = 0.0001) (Figure 1C).

Reduced food intake and increased water intake with a long-term HPD. The HPD-fed mice had a significant decrease in weekly measured food intake and increased water intake (**Supplemental Figure 1**). The BioDAQ analysis showed that the HPD-fed mice had a significant decrease in total cumulative food intake during the 24-h period compared with CD-fed mice (2.31 ± 0.13 compared with 3.49 ± 0.26 g; P = 0.023) (**Figure 2**A). Feeding behavior was further analyzed by dividing a 24-h period into 12-h increments: light phase (0600–1759) and dark phase (1800–0559). Bout duration, bout frequency, meal duration, meal frequency, meal size, and eating rate were analyzed in each group of mice during each of the 2 phases. During the dark phase, HPD-fed mice had a significant decrease in bout duration (99.08 ± 21.54 compared with 180.1 ± 22.52 min; P = 0.0008) (Figure 2B). Bout frequency was significantly decreased during the dark phase in the HPD group (74.29 ± 7.46 compared with 96.50 ± 4.24; P = 0.02) (Figure 2C). HPD-fed mice had a significant decrease in meal duration in the dark phase (134.63 ± 30.1 compared with 217.45 ± 26.43 min/meal; P = 0.01) (Figure 2D), whereas no significant difference was found in meal frequency between the 2 groups (Figure 2E). A significant decrease in meal size was found in the HPD group compared with the CD group (2053 ± 316.70 compared with 1230 ± 163.50 mg/meal; P = 0.01) (Figure 2F). A significant decrease in eating rate was observed between the HPD-fed mice compared with the CD-fed mice (1.71 ± 0.23 compared with 3.25 ± 0.36 mg/min; P = 0.0032) (Figure 2G).

Water intake increased significantly during the dark phase in HPD-fed mice compared with CD-fed mice (2.80 ± 0.32 compared with 1.60 ± 0.29 g; P = 0.0027) (**Figure 3**A). Mean water intake significantly increased in HPD-fed mice compared

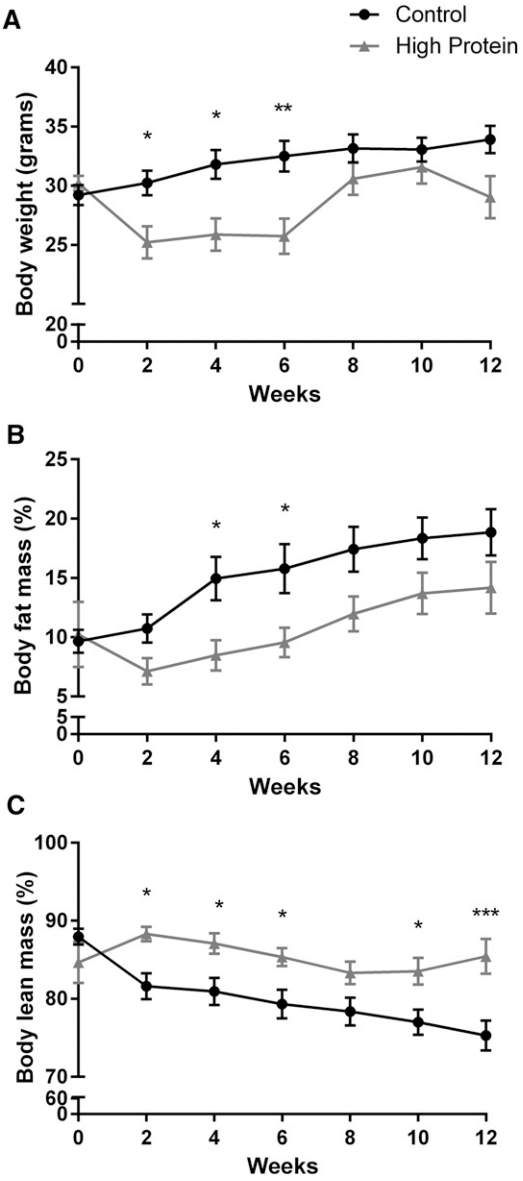

FIGURE 1 Body weight (A), body fat mass (B) and body lean mass (C) analysis of HPD- and CD-fed mice during the 12-wk period in 2-wk intervals. We used unpaired *t* tests to perform comparisons between HPD- and CD-fed mice. Values are means ± SEMs; HPD group, n = 8; CD group, n = 10. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. CD, control diet; HPD, high-protein diet.

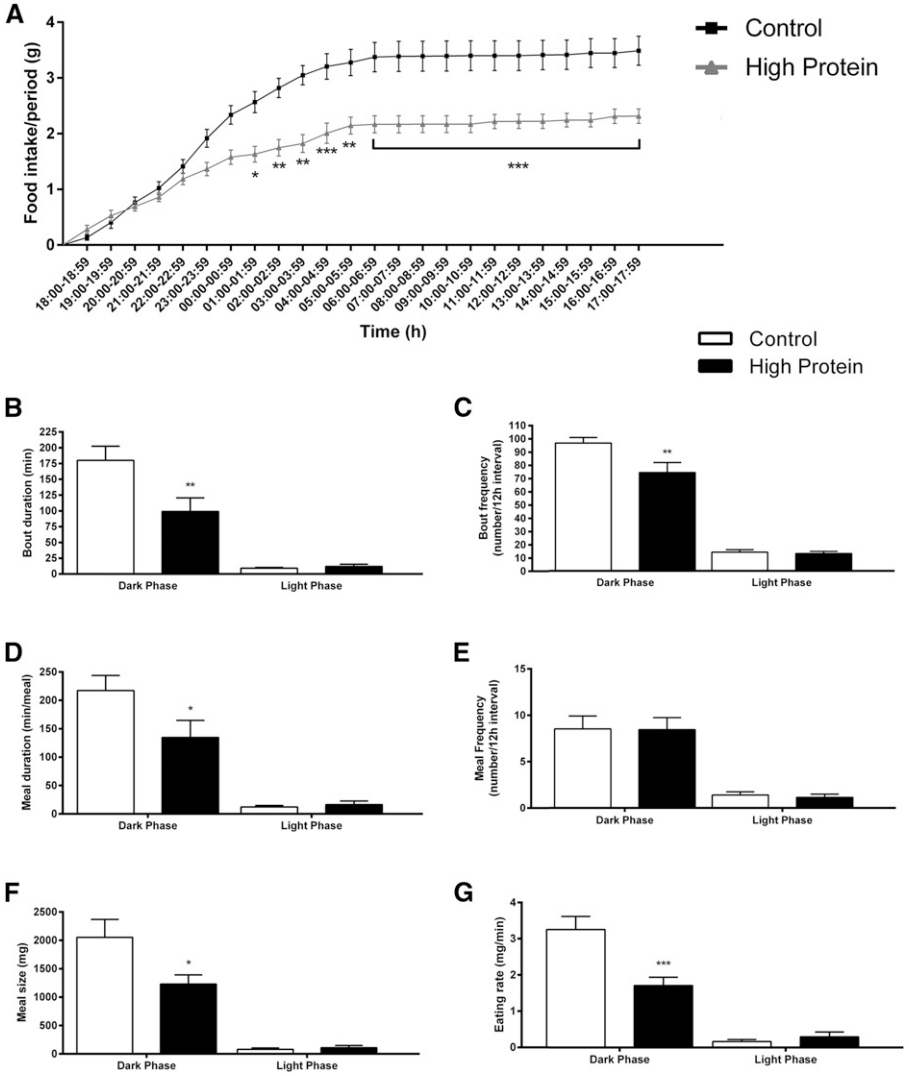

FIGURE 2 Food intake analysis between HPD- and CD-fed mice. (A) Food consumption data for HPD- and CD-fed mice expressed as 24-h average values over 48 h of uninterrupted recording; cumulative food intake was calculated by using 2-h intervals. (B) Bout duration, (C) bout frequency, (D) meal duration, (E) meal frequency, (F) meal size, and (G) eating rate analyzed between the dark and light cycles. We used unpaired *t* tests to perform comparisons of food consumption data. We used 2-factor ANOVA analysis to compare bout duration, bout frequency, meal duration, meal frequency, meal size, and eating rate values during the dark and light phases between HPD- and CD-fed mice. Values are means ± SEMs; HPD group, $n = 8$; CD group, $n = 10$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. CD, control diet; HPD, high-protein diet.

with CD-fed mice during light (0.06 ± 0.01 compared with 0.03 ± 0.01 g; $P = 0.003$) and dark (0.06 ± 0.003 compared with 0.04 ± 0.005 g; $P = 0.034$) phases (Figure 3B). No significant difference was found in the number of bouts (Figure 3C) and in the time spent drinking (Figure 3D) between the 2 groups during the dark and light phases.

Effects of long-term treatment with HPD on metabolic rate and energy expenditure. TEE was lower in HPD-fed mice than in CD-fed mice (**Figure 4**A); the averaged values were significantly lower during both the dark phase (0.48 ± 0.01 compared with 0.55 ± 0.01 kcal/h; $P = 0.003$) and the light phase (0.37 ± 0.01 compared with 0.42 ± 0.01 kcal/h; $P = 0.03$) (Figure 4B). Twenty-four-hour feed-deprived HPD-fed mice had lower TEE values than CD-fed mice (Figure 4C), and the averaged values were significantly lower during the dark phase (0.34 ± 0.01 compared with 0.44 ± 0.03 kcal/h; $P = 0.03$) (Figure 4D), similar to mice fed in ad libitum conditions. RQ values were lower in HPD-fed mice than in CD-fed mice (Figure 4E), and the averaged values were significantly lower during both the dark phase (0.80 ± 0.02 compared with 0.87 ± 0.03; $P = 0.01$) and the light phase (0.74 ± 0.01 compared with 0.82 ± 0.02; $P = 0.007$) (Figure 4F). However, in feed-deprived conditions, the differences in RQ values between HPD- and CD-fed mice were not significantly different during either the dark or light phase (Figure 4G, H) compared with mice fed ad libitum. Interestingly, the VH_2O values were significantly higher in the HPD-fed mice than in the CD-fed mice (Figure 4I); the averaged values were significantly higher during both the dark phase (0.32 ± 0.03 compared with 0.23 ± 0.02 mL/min; $P = 0.01$) and the light phase (0.24 ± 0.01 compared with 0.14 ± 0.01 mL/min; $P = 0.006$) (Figure 4J). However, VH_2O values were not significantly different between HPD-fed and CD-fed mice during feed-deprived conditions (Figure 4K, L).

When examining physical activity, no significant differences in total activity index, coarse activity index, fine activity index, and mean locomotion speed were observed between the 2 HPD- and CD-fed mouse groups (**Supplemental Figure 2**A–D). Similarly, under 24-h feed-deprived conditions, no significant difference was shown in the 2 groups of mice for physical activity, as measured by the total activity index, coarse activity index, fine activity index, and mean locomotion speed (**Supplemental Figure 3**A–D). Behavioral analysis performed by using the Promethion metabolic system showed a significantly lower interaction with the food hopper (significant food intake) in the HPD-fed group during the dark phase (time budget analysis, as a percentage of total time: 8.50% ± 1.40% compared with 13.40% ± 1.80%; $P = 0.01$) (**Supplemental Figure 4**A). However, no significant difference was observed between the 2 mouse groups, during both the light and dark phases, in the

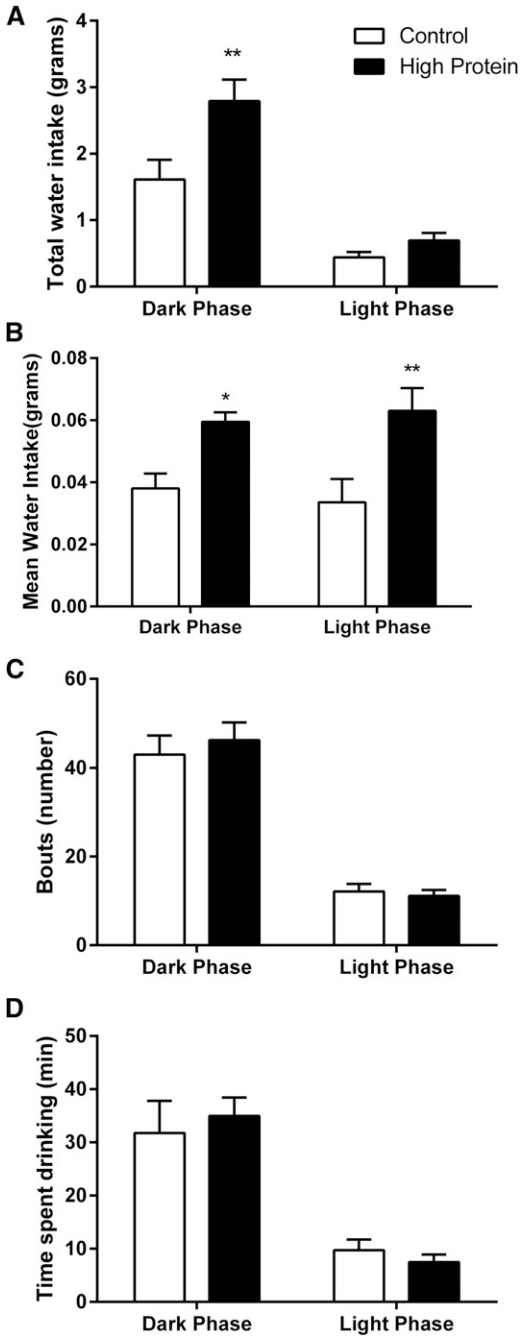

FIGURE 3 Water intake and drinking behavior variables between HPD- and CD-fed mice. Total water intake (A), mean water intake (B), drinking bouts (C), and time spent drinking (D) analyzed between the dark and light cycles. Values are means ± SEMs; HPD group, $n = 8$; CD group, $n = 10$. $^{*}P < 0.05$, $^{**}P < 0.01$. CD, control diet; HPD, high-protein diet.

following analyzed behavioral variables: interaction with food hopper (no significant food intake), interaction with water hopper (significant water intake), interaction with water hopper (no significant water intake), and time in habitat, long lounge (period of inactivity >5 min), and short lounge (period of inactivity >5 min) (Supplemental Figure 4B–G).

Altered feed-deprived and postprandial plasma metabolic hormone concentrations. In HPD-fed mice, in comparison to CD-fed mice, plasma concentrations of leptin, GLP-1, and glucagon hormones were significantly altered: HPD-fed mice had lower leptin during both feed-deprived (41.31 ± 11.60 compared with 3041 ± 683 pg/mL; $P = 0.0004$) and postprandial (112.5 ± 102.0 compared with 8273 ± 1415 pg/mL; $P < 0.0001$) conditions compared with CD-fed mice (**Figure 5**B). HPD-fed mice had significantly lower GLP-1 than CD-fed mice during feed-deprived (5.664 ± 1.44 compared with 21.31 ± 1.26 pg/mL; $P = <0.0001$) and postprandial (6.54 ± 2.13 compared with 50.62 ± 11.93 pg/mL; $P = 0.0037$) conditions (Figure 5D). In addition, postprandial glucagon concentrations were significantly higher in HPD-fed mice than in CD-fed mice (102.3 ± 35.8 compared with 30.99 ± 6.58 pg/mL; $P = 0.043$) (Figure 5E). Finally, the concentrations of active ghrelin, insulin, and PYY showed no significant differences between the 2 experimental mouse groups in either feed-deprived or postprandial conditions (Figure 5A, C, F).

Discussion

HPD treatments are largely used in humans to achieve body weight and fat mass loss and to maintain or increase muscle mass (1–6). HPD strategies are also frequently used in patients undergoing bariatric surgery to maintain lean body mass and to prevent weight gain (36). However, previously published studies reported that HPDs induce phenotypic effects in humans that are limited to an initial period of ~6 mo, after which time the subjects regain body weight (15, 16). To our knowledge, the effects of long-term HPD treatments on energy intake, energy expenditure, physical activity, and metabolic hormones have never been evaluated. Consequently, we analyzed 2 different groups of age-matched mice that were fed an isocaloric HPD or CD for 12 wk to elucidate their metabolic responses to a long-term HPD regimen.

Our results showed that long-term HPD treatment induced an initial decrease in body weight and fat mass for only 2–6 wk, during which time the mice were observed to have a reduced energy intake while maintaining body lean mass. Several published studies (4, 7, 8, 11, 23, 32, 37–39) that used short-term HPD treatments also showed similar trends in body weight loss in rodents and in humans. Body lean mass was reported to be potentially preserved by elevated plasma concentrations of amino acids that could promote muscle growth and preservation (40–44). HPDs decreased muscle protein degradation in a murine model of muscular dystrophy (40) while increasing muscle endurance, especially after physical exercise, in humans (42, 44, 45). However, physical activity was not found to be increased in the HPD-fed mouse group as described previously (31). Earlier observations in HPD-fed rats suggested that the main determinant of reduced energy intake was poor palatability (46); however, these studies examined the effects of an HPD switch for only a few hours. Other rodent and dog studies that used flavor testing, behavioral satiety sequence, and taste reactivity showed that the reduced energy intake was due to a specific mechanism by which a protein meal enhances satiety and suppresses food intake (8, 10, 20, 22, 47).

Although previous studies examined calorimetry and energy expenditure in feed-deprived conditions by using a single HPD intake for up to a 24-h time interval (26–30), our study analyzed the long-term effects of an HPD. A continuous metabolic analysis in both ad libitum and feed-deprived conditions during a 48-h period showed lower TEE, thus suggesting that high protein intake lowers metabolic rate and reduces oxidation of carbohydrates, which is consistent with the lower carbohydrate intake (48, 49). The lower metabolic rate found in our HPD-fed mice

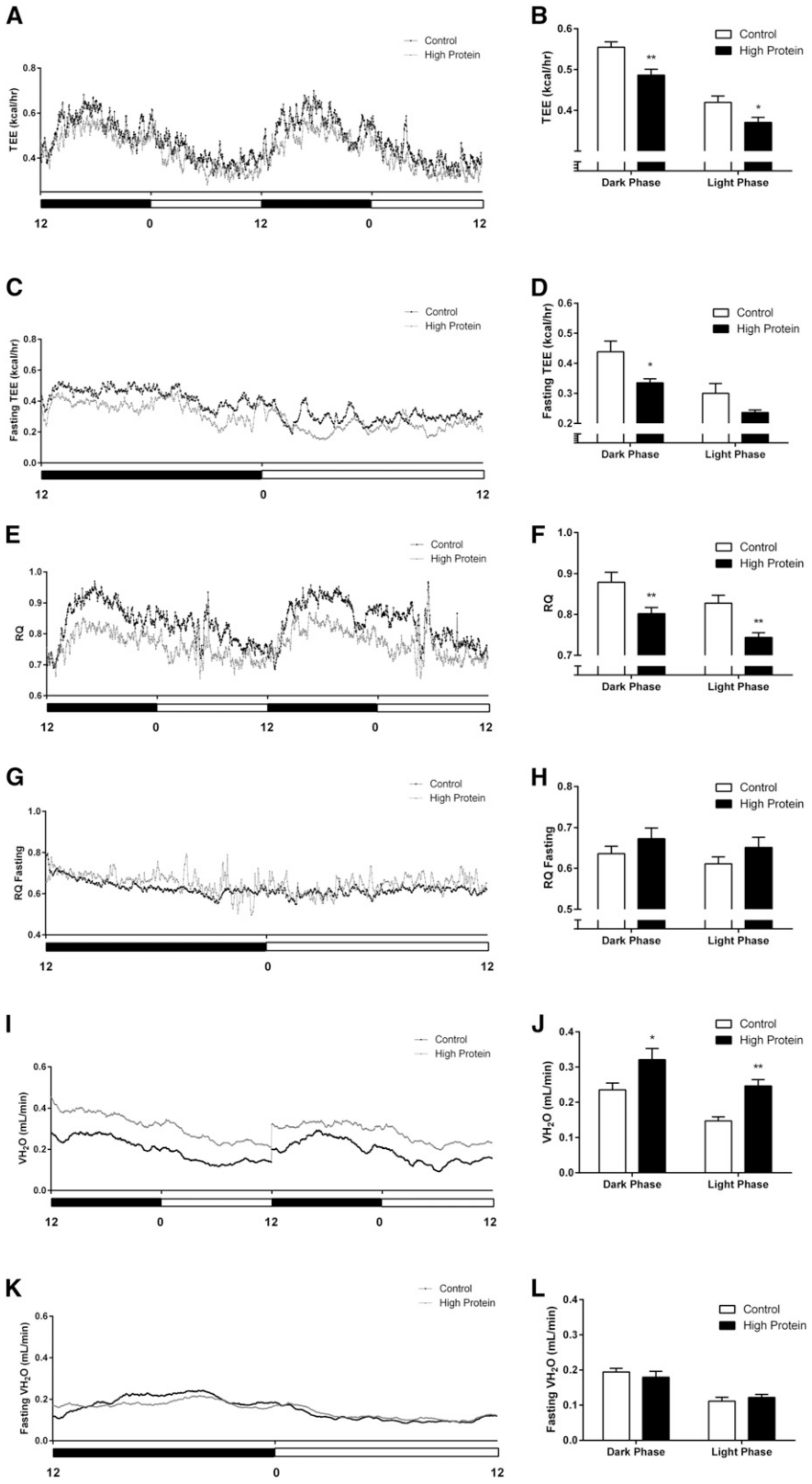

FIGURE 4 Analysis of respirometry and calorimetry data between HPD- and CD-fed mice. TEE (A) and dark phase and light phase averages of TEE (B) between HPD- and CD-fed mice; TEE (C) and dark phase and light phase averages of TEE (D) between HPD- and CD-fed mice in feed-deprived conditions; RQ (E) and dark phase and light phase averages of RQ (F) between HPD- and CD-fed mice; RQ (G) and dark phase and light phase averages of RQ (H) between HPD- and CD-fed mice in 24-h feed-deprived conditions; VH_2O (I) and dark phase and light phase averages of VH_2O (J) between HPD- and CD-fed mice; VH_2O (K) and dark phase and light phase averages of VH_2O (L) between HPD- and CD-fed mice in 24-h feed-deprived conditions are shown. Values in panels B, D, F, H J, and L are means ± SEMs; HPD group, $n = 8$; CD group, $n = 10$. $^*P < 0.05$, $^{**}P < 0.01$. CD, control diet; HPD, high-protein diet; RQ, respiratory quotient; TEE, total energy expenditure; VH_2O, water vapor.

was also correlated with decreased energy intake due to the satiating effect of an HPD. In several human clinical trials that used short-term high-protein interventions, an increase in energy expenditure of 0.8–22% was observed (26–30, 50, 51). These clinical studies indicated that a high protein intake increases thermogenesis and energy expenditure (50). However, these TEE increases were found only during the first 2 wk, whereas in a longer-term study of 6 wk, no significant differences were observed (52, 53). However, these human studies included only feed-deprived subjects who received a single high-protein meal and an analysis that lasted for intervals of only 30 min to 24 h (54). Kim et al. (31) showed in a 12-wk study in mice that energy expenditure was lower in the low-fat, high-protein diet group, even though the values did not reach significance. In our study,

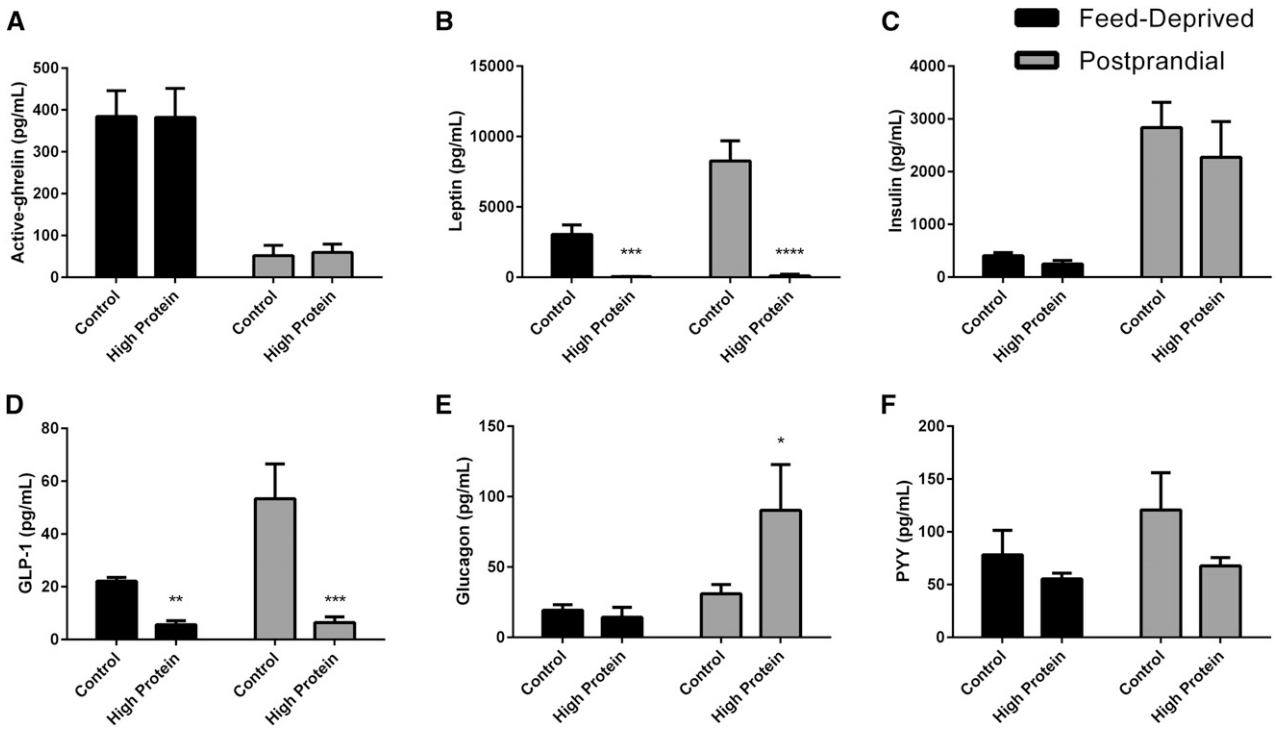

FIGURE 5 Orexigenic and anorexigenic metabolic plasma hormone analyses of active ghrelin (A), leptin (B), insulin (C), GLP-1 (D), glucagon (E), and PYY (F) plasma concentrations measured in either feed-deprived or postprandial conditions between HPD- and CD-fed mice. Values are means ± SEMs; HPD group, $n = 8$; CD group, $n = 10$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. CD, control diet; GLP-1, glucagon-like peptide 1; HPD, high-protein diet; PYY, peptide YY.

HPD-fed mice showed a reduced TEE, suggesting a metabolic adaptation response to the long-term HPD, which induced a reduction in energy intake as was shown in a rat model (51). We observed lower RQ values in the HPD-fed group in ad libitum feeding conditions. The lower RQ values observed in HPD-fed mice presumably reflect the lower carbohydrate content of the HPD used in this study than in the CD; this was verified by the similar RQ values observed in feed-deprived HPD- and CD-fed mice.

We observed a significant increase in total water intake and mean water intake in the HPD mouse group. HPDs are acidogenic and increase the production of ammonia solutes that are excreted with water (55, 56). Previous human studies have shown that an increase in water consumption can promote satiety, thus reducing energy intake and inducing body weight loss (57, 58). The increased water intake measured in the HPD-fed mice might be related to the elevated rates of urea and creatinine as postulated previously (59). Our results are in concordance with other studies performed in mice that reported similar increases in water consumption during an increase in dietary protein (60–62).

Dietary proteins have been shown to induce the release of anorexigenic and orexigenic hormones that modulate neuronal pathways involved in the regulation of appetite and satiety and that mediate the metabolic responses to nutrient availability (12). Our data show that key metabolic hormones, such as leptin, GLP-1, and glucagon, measured at study week 12, were significantly altered in HPD-fed mice compared with CD-fed mice in both feed-deprived and postprandial conditions. HPD-fed mice had significantly reduced leptin concentrations during both feed-deprived and postprandial conditions. Generally, plasma concentrations of leptin have been shown to be proportional to body fat mass, because leptin plays a major physiologic function in informing the central nervous system about the amount of energy that is stored to regulate satiety and energy expenditure (63–66). Schwarz et al. (32) also documented a similar decrease in plasma leptin after a 12-wk high-protein intake in mice, whereas significantly higher concentrations were reported in high-fat-diet–fed mice. In addition, Binder et al. (67) showed that a higher consumption of dietary protein leads to an increased sensitivity to leptin. Other studies that examined the effects of HPDs also reported lower plasma leptin concentrations (4, 9). Previous published data of 24-h studies in human subjects showed that an acute increase in oral protein intake leads to higher GLP-1 plasma concentrations (67–69). Although GLP-1 is considered to be an anorexigenic hormone, we observed significantly lower concentrations of GLP-1 in both feed-deprived and postprandial conditions in HPD-fed mice; therefore, it is unlikely that GLP-1 is involved in the reduction in food intake induced by an HPD. Interestingly, in our current study, plasma glucagon concentrations were significantly higher in HPD-fed mice only in postprandial conditions.

In conclusion, this study shows that a 12-wk HPD treatment significantly reduced energy intake, increased body lean mass, and altered the metabolic hormone profile in mice. HPD induced changes in body phenotype that were associated with a significant increase in satiety and water intake and a decrease in oxygen consumption, carbon dioxide production, RQ, and TEE. Furthermore, HPD-fed mice had significantly lower feed-deprived and postprandial leptin and GLP-1 concentrations, although postprandial glucagon concentrations were elevated. However, future studies are needed to elucidate the underlying mechanisms by which dietary proteins regulate oxygen consumption, carbon dioxide production, and energy expenditure. An analysis of the genes or transcriptome involved in lipid metabolism and adipogenesis could elucidate the mechanisms underlying the adjustment in energy expenditure leading to the enhanced lean mass phenotype observed in the HPD-fed mice throughout the study.

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