

A High-Whey-Protein Diet Reduces Body Weight Gain and Alters Insulin Sensitivity Relative to Red Meat in Wistar Rats^{1,2}

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ABSTRACT A high-protein diet can reduce body weight and increase insulin sensitivity, but whether the type of dietary protein affects these outcomes is unknown. We hypothesized that feeding insulin-resistant rats a high-protein diet (32%) containing whey protein concentrate (WPC) would reduce body weight and tissue lipid levels and increase insulin sensitivity more than a diet containing red meat (RM). Rats were fed a high-fat diet (300 g fat/kg diet) for 9 wk, then switched to a diet containing either 80 or 320 g protein/kg diet, provided by either WPC or RM, for 6 wk ($n = 8$). The rats were then killed after overnight food deprivation. High dietary protein reduced energy intake ($P < 0.001$) and visceral ($P < 0.001$), subcutaneous ($P < 0.001$), and carcass fat ($P < 0.05$). Increasing the dietary density of WPC, but not of RM, reduced body weight gain by 4% ($P < 0.001$). Dietary WPC also reduced plasma insulin concentration by 40% ($P < 0.05$) and increased insulin sensitivity, compared to RM ($P < 0.05$). These findings support the conclusions that a high-protein diet reduces energy intake and adiposity and that whey protein is more effective than red meat in reducing body weight gain and increasing insulin sensitivity. *J. Nutr.* 134: 1454–1458, 2004.

KEY WORDS: • whey protein • red meat • protein density • weight gain • insulin sensitivity

The Western diet, characterized by elevated intakes of red meat, saturated fat, and refined carbohydrates and low intakes of fiber and calcium, is associated with increased risk for insulin resistance and obesity (1). At present, energy restriction is the most effective way for individuals with insulin resistance, obesity, and/or non-insulin dependent diabetes mellitus to improve their glucose control and plasma lipid profile and lose weight (2). However, weight loss by this method also reduces satiety and increases appetite, which makes adherence to an energy-restricted diet difficult (3). Altering the macronutrient composition of the diet may be a more effective way to increase the rate of fat and weight loss and increase insulin sensitivity. Increasing the density of protein in the diet reduces energy intake by increasing satiety (4) and increases total energy expenditure by means of the increased thermogenesis associated with protein digestion (4). Free consumption of a high-protein diet reduces energy intake and increases the rate of weight and fat loss in obese subjects, compared to a low-protein diet (5). However, because these studies used a mixed-

protein meal, it is not clear whether protein type (red meat, dairy, or vegetable protein) affects these outcomes.

Animal studies show that red meat (RM)⁴ in the diet increases body weight more than dairy protein [casein or whey protein concentrate (WPC)] (6,7). Increased dietary density of RM is also positively associated with weight gain in rats (8,9). Conversely, moderate (10–12) or high (9) dietary density of WPC reduces body weight in rats. Because the reported studies were conducted with predominately young growing rats, it is not known whether the dietary proteins similarly affect body weight in mature insulin-resistant rats. Consumption of a high-fat diet (30% by wt) for at least 3 wk induces insulin resistance in outbred Wistar rats (13). Insulin resistance in rats is marked by increased visceral fat and muscle triglyceride accumulation, whole body and skeletal muscle insulin resistance, and hyperinsulinemia (13,14). We hypothesized that feeding insulin-resistant Wistar rats a high-protein diet (32%) containing WPC would reduce weight gain and tissue lipid levels and increase insulin sensitivity more than a diet containing RM as the protein source.

MATERIALS AND METHODS

Animals. Male Wistar rats ($n = 32$; age = 10 wk; body wt = 367 ± 6 g) were obtained from the Laboratory Animal Services Branch,

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⁴ Abbreviations used: RM, red meat; T₃, triiodothyronine; T₄, tetraiodothyronine; WPC, whey protein concentrate.

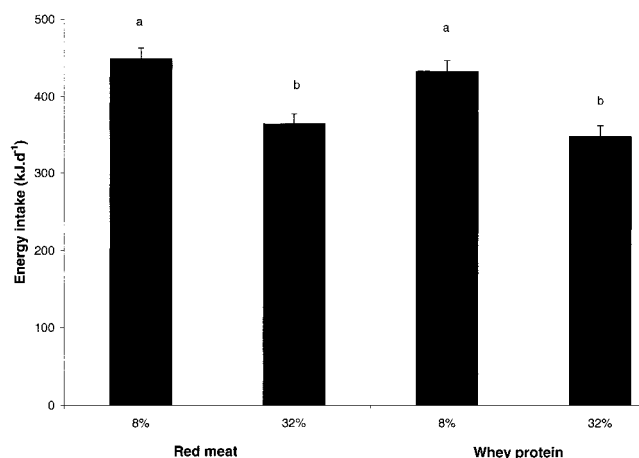


FIGURE 1 Effect of diets that differ in protein type and density on the energy intake of Wistar rats. Values are means \pm SEM, $n = 8$. Means without a common letter differ, $P < 0.05$.

Adelaide University. The rats were housed in wire cages to minimize coprophagy and were maintained in an air-conditioned environment at $23 \pm 2^\circ\text{C}$ with a 12-h light–dark cycle.

The rats were fed a high-fat (300 g fat/kg diet) AIN-93–based diet for 9 wk. Blood was then taken from the tail vein after overnight food deprivation (14 h), and the insulin and glucose concentrations were measured to ensure that the rats were insulin resistant. The rats were then separated randomly into 4 dietary treatment groups ($n = 8/\text{group}$) and fed test diets for 6 wk. The low-protein diets contained 8% WPC or RM (80 g protein/kg diet), and the high-protein diets 32% RM or WPC (320 g/kg diet). The rats were housed individually, and individual food intake was measured daily.

At the conclusion of the study, the rats were deprived of food overnight, then anesthetized with 4% fluothane:oxygen (v:v) and killed by exsanguination from the abdominal aorta. The visceral (mesenteric, epididymal, and retroperitoneal) and subcutaneous fat pads were removed and weighed. The remaining internal organs, skin, feet, head, and tail were then removed, leaving the carcass (muscle mass and skeleton). The carcass was dried and ground to obtain a consistent sample for fat, protein, and calcium analysis. All experimental procedures using rats were approved by the Commonwealth Scientific and Industrial Research Organization, Health Sciences and Nutrition Animal Ethics Committee, and the University of Adelaide Animal Ethics Committee.

Diets. The high-fat diet contained 300 g fat (1:1 sunflower seed oil:beef fat), 510 g sugar, 120 g casein, and 20 g fiber as α -cellulose per kg of diet. Choline, cysteine, minerals, and vitamins were added as described in AIN-93 (15). The experimental diets varied in protein type and density between treatment groups. Diets contained either 80 or 320 g protein/kg, provided by barbecued kangaroo red muscle meat (780 g protein/kg RM) or whey protein concentrate (790 g protein/kg WPC) [protein composition and source as outlined in Belobrajdic et al. (9)]. The protein density of the diets was adjusted using carbohydrate (3:2 cornstarch:sugar). The fat content of the diets was 200 g/kg diet. The majority of the dietary fat was provided by sunflower seed oil and beef fat (1:1), with the remainder provided by RM or WPC. Minerals provided by the various protein types were balanced in all diets to a constant level, as described in AIN-93 (15). The diets provided low concentrations of calcium (1 g/kg) and fiber (20 g as α -cellulose/kg) to simulate the Western diet.

Carcass analysis. Each carcass was freeze-dried, minced, and ground with a hammer mill to provide a fine powder for analysis. The carcass fat content was assayed gravimetrically, following extraction with 2:1:1 chloroform:methanol:water as described by Folch et al. (16). The carcass protein content was quantified by the Dumas method (17), using a Carlo Erba NA 1500 nitrogen analyzer. The quantity of bone in the carcass was calculated from the calcium value.

Carcass samples were digested with acid, then analyzed for calcium by atomic absorption spectrometry (Varian).

Blood chemistry and hormone analysis. The plasma insulin concentration was determined by RIA (Linco Research) at 9 wk and by ELISA (ALPCO) at the end of the study. The plasma glucose concentration was determined spectrophotometrically using a commercially available kit (Gluko-quant, Roche). Concentrations of corticosterone and the thyroid hormones triiodothyronine (T_3) and tetraiodothyronine (T_4) were measured by RIA (Brahms). The insulin resistance of the rats was quantified using the insulin:glucose ratio, which has been used previously as an index of insulin sensitivity in rats (18,19) and other species (20) and which correlates strongly with measures obtained by the euglycemic-hyperinsulinemic clamp technique in humans (21).

Statistics. Statistical analyses were performed using SPSS 10.0 for Windows (SPSS). The effects of protein type and density were assayed by two-way ANOVA followed by Tukey's multiple comparison test. The effects of dietary protein type and density on body weight (wk 10–17) were examined by between-factor and repeated-measures ANOVA, with weight at wk 9 as a covariate. The effects of dietary protein type and density on dietary intake were examined using between-factor and repeated-measures ANOVA. Values were expressed as means \pm SEM and differences between treatment means were considered significant at $P < 0.05$.

RESULTS

Weight gain and energy intake. Energy intake was inversely related to dietary protein density. Rats fed the high-protein diets consumed 19% less energy/d than rats fed the low-protein diets ($P < 0.05$; Fig. 1). However, dietary protein type did not affect energy intake. The rats fed the 32% WPC diet gained 4 and 10% less weight than the rats fed the 8% protein diets and the 32% RM diet, respectively ($P < 0.001$; Fig. 2).

Body composition and tissue lipids. Increasing the density of protein from 80 to 320 g/kg diet decreased the weight of visceral and subcutaneous fat by 22.6% ($P < 0.05$) and 25.6% ($P < 0.001$), respectively (Table 1). Total visceral fat weight in rats fed the 32% RM and WPC diets was less than in rats fed the 8% RM and WPC diets ($P < 0.05$; Table 1). Subcutaneous fat weight was less in the 32% WPC group than in the 8% RM group ($P < 0.05$; Table 1). Most fat depots of rats in the 32% RM group did not differ from those of the other groups. Protein type did not affect kidney or liver weight.

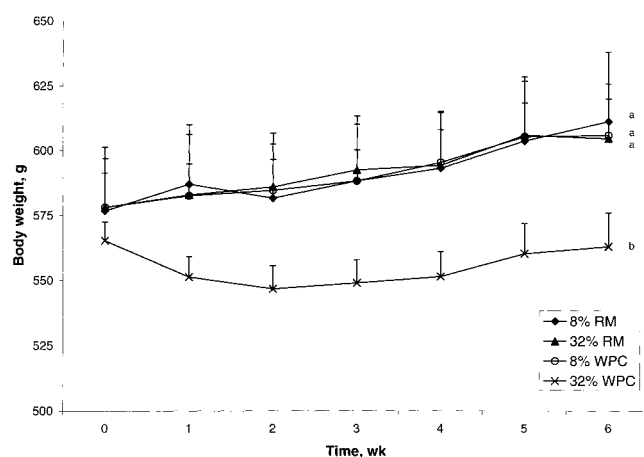


FIGURE 2 Effect of diets that differ in protein type and density on the body weight of Wistar rats. Values are means \pm SEM, $n = 8$. Means without a common letter differ, $P < 0.05$.

TABLE 1

Effect of diets that differ in protein type and density on fat pad weight and carcass composition of Wistar rats¹

| Variable | Red meat | | Whey protein | | ANOVA | | |
|--------------------------|-------------------------|------------------------|------------------------|------------------------|-----------------|---------|-------|
| | 8% | 32% | 8% | 32% | Type | Density | T × D |
| <i>g</i> | | | | | | | |
| Body weight | 594 ± 18 | 572 ± 17 | 588 ± 20 | 546 ± 18 | NS ² | NS | NS |
| Visceral fat pad | | | | | | | |
| Epididymal | 16 ± 1.2 ^a | 13 ± 0.9 ^{ab} | 15 ± 0.9 ^a | 11 ± 0.6 ^b | NS | 0.002 | 0.003 |
| Mesenteric | 15 ± 2 ^a | 11 ± 1 ^{ab} | 12 ± 0.2 ^{ab} | 9 ± 0.6 ^b | NS | 0.022 | 0.034 |
| Perinephric | 26 ± 3 ^a | 20 ± 2 ^{ab} | 26 ± 2 ^a | 16 ± 1 ^b | NS | 0.001 | 0.01 |
| Total | 57 ± 6 ^a | 45 ± 3 ^b | 53 ± 3 ^a | 36 ± 2 ^b | NS | 0.001 | 0.005 |
| Subcutaneous fat | 59 ± 8 ^a | 43 ± 5 ^{ab} | 49 ± 6 ^{ab} | 33 ± 3 ^b | NS | 0.014 | 0.038 |
| Liver | 18.2 ± 1.3 | 16.6 ± 0.8 | 17.6 ± 0.7 | 16.1 ± 13.4 | NS | NS | NS |
| Kidney | 3.1 ± 0.1 ^{ab} | 3.5 ± 0.2 ^a | 3.0 ± 0.1 ^b | 3.5 ± 0.1 ^a | NS | 0.005 | 0.046 |
| Carcass, wet | 263 ± 7 | 279 ± 6 | 265 ± 7 | 266 ± 7 | NS | NS | NS |
| Carcass, dry | 93 ± 4 | 94 ± 3 | 92 ± 3 | 87 ± 1 | NS | NS | NS |
| <i>g/kg</i> | | | | | | | |
| Carcass composition, dry | | | | | | | |
| Fat | 240 ± 10 ^a | 220 ± 10 ^{ab} | 230 ± 10 ^{ab} | 190 ± 10 ^b | NS | 0.018 | 0.044 |
| Protein | 570 ± 20 ^b | 600 ± 20 ^{ab} | 590 ± 20 ^{ab} | 640 ± 20 ^a | NS | NS | 0.049 |
| Bone | 38 ± 3 | 39 ± 3 | 32 ± 4 | 32 ± 3 | NS | NS | NS |

¹ Values are means ± SEM, *n* = 8. Means in a row with superscripts without a common letter differ, *P* < 0.05.

² Not significant, *P* > 0.05.

Increasing protein density increased kidney weight (*P* < 0.05), but did not affect liver weight (Table 1).

Dietary protein type and density affected carcass composition (Table 1). Increased dietary protein density decreased the carcass fat content by 8.6% (*P* < 0.05) and generally increased the carcass protein content by 9.4% (*P* = 0.061). The carcasses of rats in the 32% WPC group had less fat and more skeletal muscle than those of 8% RM group, but did not differ from those of the other 2 groups (Table 1). Bone density tended to be lower in rats fed WPC than in those fed RM (*P* = 0.05).

Circulating hormones and insulin sensitivity. After 9 wk of treatment with the high-fat diet, the plasma insulin concentration of rats in the present study (135 ± 15 pmol/L) was similar to that of rats fed a high-fat diet in a previous study (123 ± 15 pmol/L) (22). These insulin concentrations were 75% higher than that of rats fed a nonpurified diet (70 ± 6 pmol/L) (22).

Rats fed WPC had a lower plasma insulin concentration (128 ± 30 pmol/L) than rats fed RM (216 ± 27 pmol/L⁻¹, *P* < 0.05; Table 2). Although the plasma glucose concentration

did not differ among the groups, the insulin:glucose ratio, a measure of insulin resistance, was lower in rats fed WPC than in rats fed RM (2.4 ± 0.5 and 4.2 ± 0.5, respectively; *P* < 0.05). Furthermore, the insulin:glucose ratio was lower in the 32% WPC group, compared to the 8 and 32% RM groups (Table 2). Protein type and density did not affect the mean plasma concentration of corticosterone (422 ± 22 μg/L) or the thyroid hormones T₃ (1.1 ± 0.08 nmol/L) and T₄ (52 ± 3.6 nmol/L).

DISCUSSION

The present study showed that a high-protein diet reduced the storage of visceral, subcutaneous, and carcass fat in mature rats by up to 26% compared to a low-protein diet. This reduction in body fat due to a high-protein diet can be partially explained by a 19% decrease in energy intake. This finding is in agreement with previous studies in rats that show that protein is more satiating than carbohydrate and fat on a weight-to-weight basis (23,24). Thus, rats fed a high-protein diet consume less energy, which might otherwise be stored as

TABLE 2

Effect of diets that differ in protein type and density on plasma insulin and glucose concentration in food-deprived rats¹

| Variable | Baseline | Red meat | | Whey protein | | ANOVA | | |
|-----------------|-----------|------------------------|------------------------|-------------------------|------------------------|-------|---------|-------|
| | | 8% | 32% | 8% | 32% | Type | Density | T × D |
| Insulin, pmol/L | 156 ± 46 | 208 ± 39 | 222 ± 36 | 169 ± 39 | 92 ± 36 | 0.03 | NS | NS |
| Glucose, mmol/L | 7.1 ± 0.2 | 6.9 ± 0.5 | 7.4 ± 0.4 | 7.3 ± 0.5 | 7.3 ± 0.4 | NS | NS | NS |
| Insulin:glucose | 3.0 ± 0.8 | 4.3 ± 0.9 ^a | 4.2 ± 0.9 ^a | 3.3 ± 0.9 ^{ab} | 1.7 ± 0.9 ^b | 0.019 | NS | 0.047 |

¹ Values are means ± SEM, *n* = 8. Means in a row with superscripts without a common letter differ, *P* < 0.05.

² Not significant, *P* > 0.05.

fat. The reduction in fat storage in rats fed a high-protein diet might also be due to the metabolic cost of digesting protein. Energy expenditure increases by up to 160% following a high-protein meal, compared to a high-carbohydrate or high-fat meal (4). Although the thermic effect of food is only a small proportion of daily energy expenditure, the change reported by Crovetti et al. (4) in relation to a high-protein diet over 3 meals could equate to a considerable increase (8.4% or 0.48 MJ) in daily energy expenditure. Another beneficial effect of the high-protein diet was a sparing of lean body mass, whereby the total muscle protein of the carcass increased and total carcass fat decreased.

There is consistent evidence in the scientific literature that changes in visceral fat mass affect plasma insulin-regulated glucose homeostasis (25). In the present study, increasing the protein density of the diet reduced fat storage, but did not affect insulin or glucose concentration, or the insulin:glucose ratio, which is a reflection of insulin sensitivity (18,19). This may be explained by the differing actions of WPC and RM on metabolism. An increase in protein density reduced weight gain when WPC was the protein source, but RM had no effect. This supports a previous study that reported an inverse association between increased dietary density of whey protein and weight gain and final body weight (9). Even with moderate-protein diets based on WPC (160 to 200 g protein/kg diet), growing rats show a reduction in growth rate and final body weight, compared to rats fed an equivalent casein diet (10–12).

In the current study, rats in the 32% WPC group had increased insulin sensitivity as assessed by the insulin:glucose ratio, along with a decrease in plasma insulin concentration. These changes may be explained by the reduction in visceral fat in the rats fed the 32% WPC diet compared to rats fed the low-protein diets, because visceral obesity is strongly correlated with insulin resistance (25). These findings also suggest that WPC intake induces the mobilization and/or utilization of visceral fat.

We postulated that WPC consumption may reduce body fat storage and weight gain by stimulating the release and activity of hormones that increase metabolic rate. Thyroid hormone (T_3 and T_4) concentrations increase in subjects consuming a high-protein diet, compared to a high-carbohydrate diet (26). In addition, a diet high in tryptophan increases plasma cortisol and adrenocorticotropic hormone concentrations (27,28). The rats in the 32% WPC group consumed twice as much tryptophan as rats in the 32% RM group (200 and 100 mg/d, respectively). However, protein type and density did not affect plasma concentrations of corticosterone or thyroid hormones. Because their levels fluctuate in response to circadian rhythm, diet, and activity, a more comprehensive examination of these hormones over a 24-h period or following a meal may yield further understanding of the effects of high-protein diets on metabolism. Other components found in WPC, such as high concentrations of leucine and lipids (conjugated linoleic acid), may have influenced body weight gain and body fat storage in the present study, but were not examined further.

Increasing the density of RM in the diet did not affect weight gain. However, previous studies (8,9) reported that increased dietary density of RM is associated with increased growth in rats. In the present study, mature rats (19 wk of age) were fed experimental diets for only 42 d, whereas Parnaud et al. (8) fed young rats test diets for 100 d, and Belobrajdic et al. (9) fed 13-wk-old rats test diets for 84 d. This suggests that RM

promotes weight gain during the most active growth period in rats. Epidemiological studies support a positive relationship between RM intake and BMI. Maskarinec et al. (29) showed that a diet high in processed and red meat, fish, poultry, eggs, and fat is positively associated with BMI, independent of energy intake. In a prospective study, Newby et al. (30) used cluster analysis to show a relation between an RM and potato diet cluster and a greater increase in BMI over time, compared to a control diet (high in fruit, vegetables, reduced-fat dairy products, and whole grains and low in red and processed meat). Although the effect of RM intake could not be separated from that of potato intake, this study does suggest that a high-RM diet may increase BMI.

The current study suggests that consumption of the high-protein diet reduced energy intake, lowered fat storage, and increased the amount of skeletal muscle protein in rats. Furthermore, different protein types within a high-protein diet had differing effects on body weight, fat storage, and insulin sensitivity. Increasing the density of WPC in the diet reduced weight gain and increased insulin sensitivity by reducing plasma insulin concentration, but an increased dietary density of RM had no effect. In conclusion, a high-protein diet based on whey protein decreased weight gain, reduced fat deposition, and increased insulin sensitivity to a greater degree than a similar diet with red meat as the protein source.

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