The Reduced Energy Intake of Rats Fed a High-Protein Low-Carbohydrate Diet Explains the Lower Fat Deposition, but Macronutrient Substitution Accounts for the Improved Glycemic Control[1–3]

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ABSTRACT The metabolic effect of high-protein low-carbohydrate (HP) diets on body composition and glucose homeostasis remains incompletely understood. This study assesses the respective roles of the increased protein:carbohydrate ratio (P:C) and the resulting moderate decrease in energy intake in the metabolic effects of HP diets. Rats had free access to normal (NP; 14%) or high (HP; 53%) total milk protein isoenergetic diets, or were fed the NP diet but restricted to the energy intake of HP rats (NPr), which was 89.1 ± 9.3% that of NP rats. After 8 wk, body weight was lower in HP and NPr rats than in NP rats. In HP rats, the lower body weight was associated with a lower adipose tissue mass and a reduced proportion of large adipocytes. HP rats also had an improved oral glucose tolerance and insulin sensitivity, as assessed by the homeostatic model assessment index, compared with NPr and NP rats, and these effects were related solely to the increased P:C. These data suggest that the reduced energy intake of rats fed a high-protein, low-carbohydrate diet explains the lower fat deposition but an increased P:C per se improves glucose homeostasis. J. Nutr. 136: 1849–1854, 2006.

KEY WORDS: • *protein:carbohydrate ratio* • *body composition* • *glucose tolerance* • *insulin sensitivity*

Low-carbohydrate, high-protein, low-energy diets are widely used in weight management programs. Compared with other diets, high-protein low-carbohydrate (HP)[5] diets are reported to be effective in inducing fat loss, while sparing lean body mass (1). A growing body of evidence shows that these diets also improve glycemic control in hyperinsulinemic, obese, or diabetic subjects (2–4). However, because most of these studies were set in the context of intense energy restriction, it is difficult to analyze the respective contributions of an elevated protein:carbohydrate ratio (P:C) and energy restriction to fat loss and various metabolic benefits.

Very few studies have addressed the effects of HP diets on healthy subjects consuming regular energy levels. Increasing the P:C while maintaining a normal energy level was shown to enhance the thermic effect of food (5), decrease energy efficiency (5), and increase satiety (6), which may facilitate long-term weight loss. Data concerning the effects of HP diets on glucose homeostasis are more controversial. On the one hand, the use of euglycemic clamps with amino acid infusion suggested that amino acids reduce glucose disposal, promote insulin secretion, and impair insulin sensitivity (IS) (7,8). BCAA were shown to impair insulin signaling in hepatoma and myotubes (9,10). Other investigations, performed in both healthy and type I diabetic subjects, also suggested that the long-term consumption of a HP diet enhances hepatic glucose output and pancreatic insulin secretion, leading to an accelerated impairment of the insulin secretion capacity (7,11).

On the other hand, recent studies suggested that HP diets are beneficial to glucose control in healthy rats and type II diabetic subjects (4,12,13). In humans consuming a HP diet, moderate weight loss, associated with a reduction in visceral adipose tissue, was shown to improve glucose homeostasis (14).

In the current study, we investigated the effects of a HP diet on energy intake, body weight, body composition, and glycemic control in healthy rats. We systematically determined the respective roles of the increased P:C and the decreased energy intake in the metabolic effects associated with the HP diet. For

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[5] Abbreviations used: AT%, atom percent; AUC, area under the curve; BW, body weight; FAS, fatty acid synthase; FFM, fat-free mass; FM: fat mass; HOMA: homeostatic model assessment; HKII, hexokinase II; HP, high-protein low-carbohydrate; IS: insulin sensitivity; IST, insulin sensitivity test; NP, normal-protein; NPr, normal-protein food restricted; OGTT, oral glucose tolerance test; P:C, protein to carbohydrate ratio; SREBP-1c, sterol regulatory element binding protein-1c; TBW, total body water.

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this purpose, we fed a group of rats a normal-protein (NP) diet, restricting their energy intake to match the spontaneous energy intake of HP-fed rats.

MATERIALS AND METHODS

Animals and diets. All experiments were carried out in accordance with the guidelines of the French Committee for Animal Care, using male Wistar rats (Harlan). Rats were adapted to the laboratory conditions for 1 wk, under a reverse light-dark cycle, as previously described (12), and accustomed to the rapid consumption (<1 min) of a glucose solution given orally with a syringe. Free access to tap water was allowed. Growth was measured 3 times/wk. After the adaptation period, rats were randomly assigned on d 1 to receive one of the experimental diets for 8 wk. The composition of the diets was described previously (1). The normal-protein high-carbohydrate (NP) diet contained 140 g total milk protein and 722 g carbohydrate/kg of food. The high-protein low-carbohydrate (HP) diet contained 530 g total milk protein and 332 g carbohydrate/kg of food. NP and HP diets were isoenergetic and contained equal amounts of fat.

Study design. In the pilot experiment, Wistar rats ($n = 16$; weighing 207 ± 5 g) were randomly assigned to either the NP or HP diet. Fresh food was freely available each day at the beginning of the dark phase (0900). Food intake, corrected for spillage, was monitored daily during wk 1 and then twice each week. At wk 8, the rats were killed [sodium pentobarbital, 30 mg/kg body weight (BW), i.p.].

In the main experiment, Wistar rats ($n = 30$; weighing 219 ± 10 g) were randomly assigned to 3 groups. NP rats consumed the NP diet ad libitum. NPr and HP rats were fed the NP and HP diets, respectively, and their energy intake was matched to that of HP rats from the pilot experiment, on a daily basis for the first 2 d and then on a weekly basis (**Table 1**). To eliminate experimental week-to-week variation, the food intake data of HP rats in the pilot experiment were smoothed to generate a linear feeding design. Food, moistened to minimize spillage, was provided daily at the beginning of the dark phase. Food intake, corrected for water evaporation, was monitored to ascertain that NPr and HP rats had similar energy intakes. Oral glucose tolerance tests (OGTT) were performed after 4 and 7 wk and i.v. insulin sensitivity tests (IST) after 5 and 8 wk. Total body water (TBW) was assessed after 5 and 8 wk. Blood samples were collected at wk 5 and 7 after at least 12 h of food deprivation, dropped in prechilled tubes containing 0.7% EDTA and 0.014% aprotinin (Bayer) and then centrifuged for 10 min at 2500 × *g*. The resulting plasma was stored at −20°C until analysis. The rats were killed after 8 wk, body composition was determined, and tissues samples were collected.

Body composition. At wk 8, after anesthesia, the liver and the right gastrocnemius muscle were collected from anesthetized rats and stored at −80°C until analysis. The interscapular brown fat pad and the epididymal, retroperitoneal, and subcutaneous white fat pads were carefully removed and weighed. The skin and the other abdominal and thoracic organs were discarded and the distal parts of the limbs, head, and tail were severed, to determine the weight of the "stripped" carcass.

TBW was assessed using deuterated water dilution as follows. Blood was withdrawn before and 120–170 min after an injection of 40 mg ^2H_2O/kg i.p. [1 mL/kg ^2H_2O, 4.064 atom percent (AT%)] (15). Plasma deuterium enrichment was determined using a multiflow system (Micromass) coupled to an isotope ratio MS (Isoprime, Micromass). TBW was calculated on the basis of simple isotopic dilution, without any correction factor (16). The fat-free mass (FFM) was estimated from TBW by assuming a constant coefficient of hydration of 72%. Fat mass (FM) was calculated as body weight minus FFM.

The size distribution of adipocytes was measured on osmium-fixed retroperitoneal fat pads as described by Hirsch (17), using a 116-class (40 nm–2 mm) laser granulometer (Beckman Coulter LS 230). Because it was shown previously that many of the particles smaller than 20 μm consisted of cellular debris, osmium-fixed free lipid droplets, and collagen particles (18), and because particles larger than 410 μm were seldom detected, only particles in the 20–410 μm range were selected for subsequent analysis.

Glucose homeostasis. The OGTT was performed after at least 12 h of food deprivation. Glucose (1 g/kg BW, 50% solution in water) was administered orally and blood glucose concentrations were measured at indicated time points. Blood was taken with a 26-gauge needle from the veins of the paw, while rats were lightly restrained. This sampling method induced little or no stress and did not affect blood glucose per se (data not shown).

The IST was performed after at least 12 h of food deprivation. Plasma glucose decay was monitored over 20 min after an insulin injection into a lateral tail vein (0.7 nmol/kg BW, i.v. bolus) (19). Blood samples were collected from the veins of the paw.

Biochemical measurements. Unless otherwise noted, all chemicals were obtained from Sigma (France). Blood glucose concentrations were measured using a portable refractometer (Glucometer, Bayer Diagnostics). Plasma insulin was detected using a solid phase 2-site enzyme immunoassay (Mercodia Rat Insulin). The homeostatic model assessment (HOMA) index was calculated as follows (20): HOMA = Insulin (pmol/L)·Glucose (mmol/L)/22.5. Plasma triglycerides levels were determined using an enzymatic assay (Triglycerides kit, BioMérieux) on a Mascott Plus robot (Lisabio).

Measurement of gene expression. We measured the hepatic expression of fatty acid synthase (FAS), the rate-limiting enzyme of hepatic lipogenesis, and that of the transcription factor sterol

TABLE 1

Daily energy intake of NP, HP, and NPr rats in the pilot and main experiments[1]

	Pilot experiment Energy intake		Energy ration[2]	Main experiment Energy intake		
	NP	HP	HP and NPr	NP	NPr	HP
d			*kJ/d*			
1	351 ± 37[a]	226 ± 18[b]	224	ND	224 ± 0	223 ± 0
2	345 ± 30[a]	279 ± 19[b]	253	331 ± 22[a]	253 ± 0[b]	250 ± 5[b]
3–6	335 ± 35[a]	296 ± 45[b]	298	321 ± 21[a]	288 ± 15[b]	282 ± 19[b]
7–13	356 ± 53[a]	287 ± 61[b]	298	334 ± 34[a]	292 ± 18[b]	295 ± 4[b]
14–20	341 ± 43[a]	304 ± 44[b]	313	350 ± 30[a]	308 ± 9[b]	296 ± 7[b]
21–27	348 ± 40[a]	310 ± 41[b]	313	332 ± 37[a]	302 ± 16[b]	302 ± 14[b]
28–33	350 ± 23[a]	318 ± 52[b]	328	287 ± 37	282 ± 42	281 ± 41
34–56	320 ± 13	288 ± 10	328	341 ± 32[a]	312 ± 23[b]	307 ± 23[b]

[1] Values are means ± SD, $n = 8$ (pilot) or 10 (main). Within an experiment, means in a row with superscripts without a common letter differ, $P \leq 0.05$.

[2] To eliminate experimental week-to-week variation, the food intake data of HP rats in the pilot experiment were smoothed to generate a linear feeding design.

regulatory element binding protein 1c (SREBP-1c), a key mediator of insulin action on FAS expression (21). We investigated muscle tissue expression of hexokinase II (HKII), the first enzyme involved in muscle glucose utilization, and that of SREBP-1c, because it was recently reported to regulate HKII expression (22).

Total muscle and liver RNA were extracted using TRIzol® reagent (Invitrogen,). The total RNA content was determined by a fluorimetric assay (Ribogreen RNA Quantification kit, Molecular Probes). Gene expression analysis was performed with a light-Cycler (MyiQ Real-Time PCR Detection System, Roche Diagnostics) using SYBR GreenI DNA binding dye (Eurogentec, RT-SN10–05NR). cDNA was synthesized from 2 μg RNA using a PTC-200 thermocycler (MJ research). Each PCR reaction was performed in a final volume of 20 μL, containing 5 μL of the RT reaction product and 15 μL of reaction buffer, which included 8 pmol of the specific forward and reverse primers for the genes of interest (Supplemental Table 1). Ribosomal 18S RNA amplifications were used to account for variability in the initial quantities of cDNA.

Statistical analysis. The data are shown as means ± SD. Cell-size distributions were compared using the χ^2 test (Freq procedure, SAS/STAT version 8; Statistical Analysis Systems Institute). Pearson correlations were calculated using the SAS Corr procedure. All kinetics were analyzed using SAS mixed models for repeated measurements, with diet and time as independent fixed factors. Other data were analyzed using mixed models with diet as the independent fixed factor. Orthogonal contrasts were used for multiple comparisons between diets. To customize the hypothesis tests for the energy effect and the P:C effect, 2 levels of treatment were contrasted together against the third (HP and NPr vs. NP for the energy effect; NP and NPr vs. HP for the P:C effect). Differences were considered significant at $P \leq 0.05$.

RESULTS

Energy intake, growth and body composition. In the pilot experiment, the HP diet induced a 30% decrease in food intake during the first 2 d after the introduction of the diets (Table 1, $P < 0.05$), but values returned to 90% of those measured in NP rats as early as d 3. In the main experiment, food intake was similar in NPr and HP rats and lower than in NP rats ($P < 0.05$) (Table 1). At wk 8, body weight was lower in HP and NPr rats than in NP rats (**Fig. 1**). Based on TBW, body composition did not differ among the groups at wk 5. However, we found a correlation between FFM at wk 5 and the postmortem weight of the stripped carcass ($r = 0.6$, $P < 0.01$) and between FM at wk 5 and the postmortem weight of the retroperitoneal adipose tissue ($r = 0.4$, $P < 0.05$), suggesting that the differences in fat deposition were already beginning at wk 5. At wk 8, FM tended to be lower in HP rats than in NP rats ($P = 0.08$), and this difference was clearly confirmed postmortem by lower white adipose tissue weights in NP than in HP rats ($P < 0.05$) (**Table 2**). In NPr rats, the adipose tissue weight did not differ from the other groups. The stripped carcass weight, an indicator of lean body mass, did not differ between NP and HP rats, whereas it was lower in NPr rats than in NP rats. The retroperitoneal fat pad was the main contributor to the higher body weight of NP rats, whereas the subcutaneous fat pad weight did not differ among the groups.

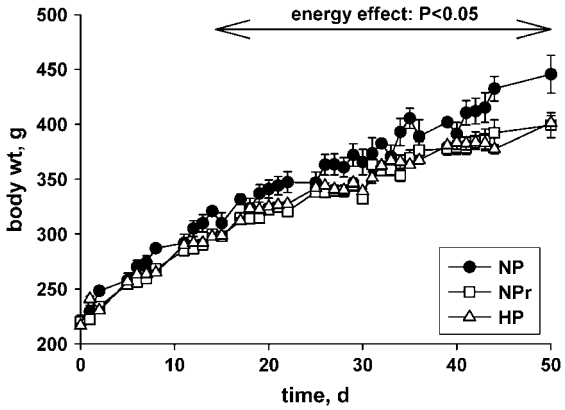

FIGURE 1 Body weights of NP, NPr, and HP rats during the 8-wk study. Values are means ± SD, $n = 10$.

The cell-size distribution of retroperitoneal fat pads clearly showed 2 cell subpopulations, with stromal cells, preadipocytes, and small adipocytes (diameter <60 μm) constituting the principal subpopulation (60–70% of cells) and larger adipocytes (diameter >60 μm) comprising the remaining 30–40% (**Fig. 2**). The overall cell-size distribution differed among the 3 groups ($P < 0.05$). The proportion of adipocytes in the 120–200 μm range was lower in HP rats than in NP and NPr rats.

Glucose homeostasis. At wk 5, blood glucose and insulin measured did not differ among the groups (data not shown). At wk 7, plasma glucose was lower in HP rats than in NP and NPr rats ($P < 0.05$), and although plasma insulin did not differ among the groups, IS (as assessed by the HOMA index) was markedly higher in HP rats than in NP and NPr rats ($P < 0.05$) (**Table 3**). Plasma triglycerides (wk 8), did not differ among the groups (data not shown).

After 4 wk, the glucose peak after the oral glucose challenge was lower in both NPr and HP rats than in NP rats (**Fig. 3**A)

TABLE 2

Body weights and post-mortem body composition of NP, HP and NPr rats at wk 8[1]

				Statistical effects	
	NP	NPr	HP	P:C	Energy
BW, *g*	431.0 ± 29.7[a]	392.2 ± 28.7[b]	388.7 ± 21.1[b]	$P < 0.05$	$P < 0.01$
Energy efficiency, *kJ feed/g gain*	97.3 ± 14.5	97.9 ± 14.9	97.6 ± 12.6	NS	NS
Stripped carcass, *g*	175.8 ± 11.5[a]	161.3 ± 14.1[b]	166.9 ± 12.4[ab]	NS[2]	$P < 0.05$
White adipose tissue, *g*	70.6 ± 17.5[a]	60.5 ± 12.4[ab]	54.1 ± 10.7[b]	$P < 0.05$	$P < 0.05$
Epididymal	15.5 ± 3.7[a]	13.42 ± 3.2[ab]	11.3 ± 2.5[b]	$P < 0.05$	$P < 0.05$
Retroperitoneal	21.6 ± 3.4[a]	18.1 ± 3.7[ab]	16.1 ± 3.2[b]	$P < 0.05$	$P < 0.05$
Subcutaneous	33.4 ± 11.5	28.9 ± 6.8	26.7 ± 6.9	NS	NS
Brown adipose tissue, *g*	0.9 ± 0.2[a]	0.8 ± 0.1[ab]	0.7 ± 0.1[b]	$P < 0.05$	$P < 0.05$

[1] Values are means ± SD, $n = 10$. Means in a row with superscripts without a common letter differ, $P \leq 0.05$.
[2] NS, $P > 0.05$.

TABLE 3

Blood glucose and insulin concentrations and the HOMA index of NP, HP, and NPr rats at wk 7[1]

				Statistical effects	
	NP	NPr	HP	P:C	Energy
Glucose, *mmol/L*	5.5 ± 0.3[a]	5.4 ± 0.2[a]	5.2 ± 0.4[b]	$P < 0.05$	NS
Insulin, *nmol/L*	0.33 ± 0.19	0.27 ± 0.19	0.18 ± 0.08	NS	NS
HOMA	75.4 ± 52.4[a]	66.2 ± 44.1[a]	42.1 ± 20.9[b]	NS	NS

[1] Values are means ± SD, $n = 10$. Means in a row with superscripts without a common letter differ, $P \leq 0.05$.
[2] NS, $P > 0.05$.

and blood glucose concentrations returned to baseline earlier in HP rats than in NP rats. At wk 4, both P:C and energy intake affected glucose kinetics ($P < 0.05$). However, the area under the glucose curve (AUC) did not differ among the groups (data not shown). In contrast, after 7 wk, glucose kinetics no longer differed among the groups (Fig. 3*B*), but the glucose AUC was lower in HP (85.1 ± 21.7 mmol·min/L) than in NPr (118.2 ± 25.7 mmol·min/L) and NP rats (117.1 ± 34.4 mmol·min/L). The P:C, but not energy intake, affected the glucose AUC.

Blood glucose kinetics in response to i.v. insulin injection, measured during the main experiment, did not differ among the groups at either 5 or 8 wk (data not shown).

Expression of SREBP1c, FAS, and HKII genes. The expression of SREBP1c, FAS, and HKII genes was investigated in liver and/or muscle tissue collected at wk 8 (**Table 4**). Hepatic SREBP1c and FAS expression were lower in HP rats than in NPr and NP rats. Muscle SREBP1c and HKII expressions did not differ among the groups but the P:C effect on HKII expression was significant.

DISCUSSION

In the context of the rising incidence of obesity and diabetes, research has focused on the role of the macronutrient balance in the diet. Although several studies have shown that diets with an increased P:C may be effective in reducing body fat and improving glucose homeostasis under various conditions (3,4,11,23,24), little is known about the underlying mechanisms. The aim of this work was to investigate the metabolic effect of a HP diet on body composition and glucose homeostasis in healthy rats and to assess the respective roles of a moderately decreased energy intake (10%) and an 800% increase in the dietary P:C in these effects.

After 8 wk, HP and NPr rats weighed 10% less than NP rats. This difference was attributable mainly to the 11% lower energy intake in HP and NPr rats because the energy efficiency did not differ between rats fed the NP and HP diets. The initial 30% reduction in food intake after the introduction of the HP diet was ascribed to both its poor palatability and the satiating effect of proteins (1,25). However, after a few days, as rats become accustomed to this new diet, the higher satiating effect of proteins accounted for the lower food intake of HP rats, as previously shown (25). Body weight was similar among HP and NPr rats, but post-mortem body compositions were qualitatively affected by the P:C. The lower body weight in HP rats was associated mainly with a reduced adiposity compared with

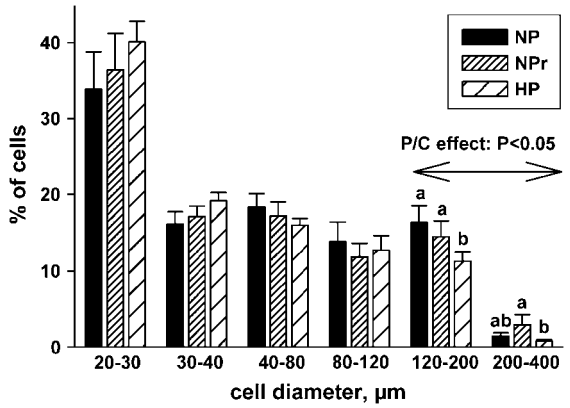

FIGURE 2 Retroperitoneal adipocytes size distribution of NP, NPr, and HP rats at wk 8. Values are means ± SD, $n = 9$. Means in a size range without a common letter differ, $P \leq 0.05$.

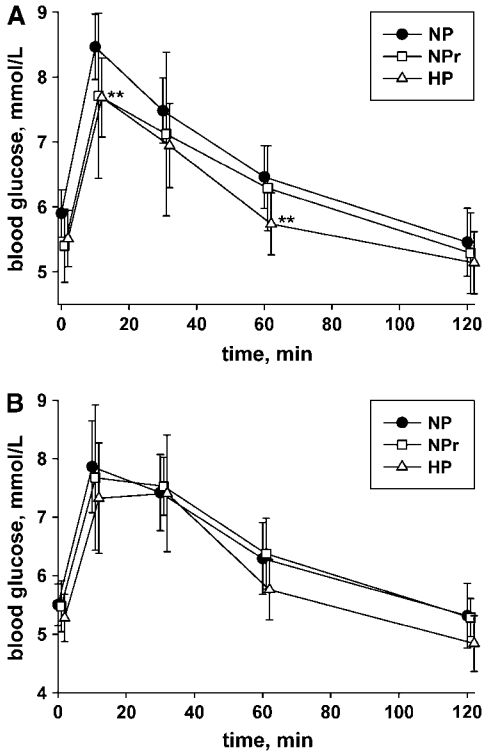

FIGURE 3 Oral glucose tolerance tests of NP, NPr, and HP rats at wk 4 (*A*) and wk 7 (*B*). Values are means ± SD, $n = 10$. **Different from NP, $P \leq 0.05$.

TABLE 4

Genes expression in NP, HP, and NPr rats at wk 8[1]

				Statistical effects	
	NP	NPr	HP	P:C	Energy
			Arbitrary units		
Liver SREBP1c	2.1 ± 0.6[a]	1.8 ± 0.7[a]	0.7 ± 0.05[b]	$P < 0.001$	$P = 0.05$
Liver FAS	0.9 ± 0.3[a]	0.8 ± 0.3[a]	0.3 ± 0.1[b]	$P < 0.05$	NS
Muscle SREBP1c	1.1 ± 0.6	1.3 ± 0.4	1.8 ± 0.3	NS	NS
Muscle HKII	1.1 ± 0.3	1.3 ± 0.8	1.9 ± 0.8	$P = 0.05$	NS

[1] Primers were designed using Oligo Explorer 1.1.0 software. The sense and antisense primers of each target gene were placed in 2 different exons of the gene.

NP rats. In NPr rats, lean body mass, as assessed by postmortem body composition, was lower than in NP rats. This resulted in a higher lean:fat mass ratio in HP rats, as previously reported (1). An increase in the diet-induced thermogenesis that was previously associated with HP diets may be responsible for the lower adiposity in HP rats compared with NPr rats (5,26). In addition, retroperitoneal adipocytes were smaller in HP rats than in NP and NPr rats, indicating a lower level of replenishment of adipose cells in HP rats. Reduced hepatic lipogenesis, as suggested by a lower hepatic gene expression of FAS and SREBP-1c, may account for the lower adiposity and the smaller proportion of large adipocytes in the retroperitoneal fat pad of HP rats. Because glucose and insulin exert similar regulatory actions on FAS in liver and adipose tissue (21), we speculate that FAS expression was also lower in the adipose tissue of HP rats, although it was not measured.

Overall, our results are in line with the data obtained during short- and long-term studies of HP diets, with or without moderate energy restriction, in both noninsulin-dependent and obese subjects (3,13,23,24). This study confirms that proteins, when substituted for carbohydrates, lower fat mass and lipogenesis levels, and preserve lean body mass.

One major outcome of the current study was the improved glycemic control in HP rats. At wk 5, there was no difference in glucose and insulin levels measured after an overnight food deprivation. Glucose tolerance (as assessed by the glucose AUC) did not differ among the groups. In contrast, after 7 wk, glucose homeostasis was markedly improved in HP rats compared with NP and NPr rats, as evidenced by OGTT and the HOMA index. The better glucose tolerance of HP rats seems to be due to a more rapid glucose clearance during the later stages of the OGTT, suggesting a higher IS. However, IST did not differ among the groups. This apparent discrepancy could be explained by a relative lack of sensitivity of this test. Indeed, the high i.v. insulin dose may have not been appropriate for discerning slight differences in sensitivity to physiological insulin. This kind of hypoglycemic test also triggers a counterregulatory response that may vary according to the diet (27). Together, these results suggest that the higher P:C is the principal contributor to the improved glycemic control in HP rats. Although the higher muscle expression of HKII in HP rats suggests that insulin action on genes involved in glucose utilization was greater in this group (22), further investigations are required to elucidate whether the improved glucose tolerance is explained mainly by a higher peripheral uptake and/or a higher suppression of hepatic glucose production.

The present results are in agreement with a previous long-term study, which demonstrated improved glucose tolerance and lower fat mass in rats fed a HP diet over a 6-mo period (12), whereas they contrast with a short-term, 10-d study that reported lower hepatic and peripheral IS and higher hepatic glucose production in rats fed the HP diet (7). The discrepancy may arise from the duration of HP feeding, because the rats may have adapted differently to the high P:C after 10 d or 6 mo. Indeed, Rossetti and co-workers (28) reported no changes in body weight and body composition. It was postulated that visceral tissue may play a key role in the onset of insulin resistance because excess visceral adipose tissue releases FFA and adipocytokines into the bloodstream, both of which are deeply involved in insulin resistance (14,29). The reduced visceral adipose tissue weight measured in HP rats at the end of the current study may explain in part the improved glucose homeostasis observed. On the other hand, HP rats preserved lean body mass, as assessed by postmortem body composition, and this may have also contribute to a better glycemic control.

Although human studies are not easily comparable to rat studies, most have reported a positive effect of HP diets on glycemic control, together with lowered body fat levels (4,13,24).

The benefits of HP diets in terms of adiposity and IS have often been linked to their lower carbohydrate content (30,31). The phenotype observed in HP rats regarding adipose tissue deposition may indeed be related to the lower carbohydrate content of the HP diet and the associated lower insulin secretion. This could explain, to some extent, the lower liver SREBP-1C and FAS expression measured in HP rats and the lower level of lipogenesis (32) (33). In examining the effects of the HP diet on glycemic regulation, the effect of a lower carbohydrate intake is less clear, and the lower insulin secretion associated with the lower carbohydrate intake does not explain the higher muscle expression of HKII. A specific role played by the protein fraction of the diet cannot be ruled out, although our study did not address this hypothesis. This would be in line with several data reports supporting a specific role of some proteins or amino acids in glycemic control. In healthy humans, long-term supplementation with an amino acid mixture improved IS (34,35), and the acute addition of a specific amino acid (glycine, arginine, or proline) improved oral glucose tolerance (36–38). Moreover, glycemic regulation in rats was shown to be affected by the nature of dietary proteins (39–41). Therefore, we cannot exclude that our results are to some extent related to the nature of the protein used in the diet formulation and that different results might have been obtained with other protein sources such as meat or soybeans.

This study provides a contribution to the debate on the influence of the macronutrient composition of a diet on body composition and glucose tolerance. Our overall findings showed

that feeding a high-protein/low-carbohydrate diet to rats reduces adipose tissue deposition, and that this effect is related both to lower energy intake and diet composition, and improves glucose homeostasis independently of energy intake. Further studies are required to confirm the suggestion that this improved blood glucose control originates from a higher peripheral IS and to address the role of candidate amino acids in insulin signaling.

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