A Reduced Carbohydrate, Increased Protein Diet Stabilizes Glycemic Control and Minimizes Adipose Tissue Glucose Disposal in Rats[1,2]

Jamie I. Baum,* Donald K. Layman,*†[3] Gregory G. Freund,* **‡ Kristen A. Rahn,† Manabu T. Nakamura,*† and Barbara E. Yudell*

*Division of Nutritional Sciences, †Department of Food Science and Human Nutrition, **Department of Pathology, ‡Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801

ABSTRACT The dietary reference intakes (DRIs) established an acceptable macronutrient distribution range (AMDR); however, few studies have evaluated differences in metabolic regulations across the DRI range. This study examined differences in glycemic regulations associated with specific ratios of carbohydrate and protein. Male rats (~200 g) were fed either a high-carbohydrate diet (CHO group: 60% of energy as carbohydrates, 12% protein, 28% fat) or a reduced-carbohydrate diet [PRO (protein) group: 42% carbohydrates, 30% protein, 28% fat]. Rats consumed 3 meals/d with energy distributed as 16, 42, and 42%. On d 25, blood and tissues were obtained after 12 h of food deprivation and at 30 and 90 min after the first meal. Before the meal, the CHO group had lower plasma glucose and insulin, reduced liver glycogen, lower expression of hepatic phosphoenolpyruvate carboxylase (PEPCK), and increased fatty acid synthase (FAS) in adipose tissue. After the meal, the CHO group had greater increases in plasma glucose and insulin, producing increased skeletal muscle phosphatidylinositol 3-kinase (PI3-kinase) activity, glucose uptake, and glycogen content, and increased adipose PI3-kinase activity, glucose uptake, and FAS. In contrast, the PRO group had limited postprandial changes in plasma glucose and insulin with reduced muscle PI3-kinase activity and glucose uptake, and no postprandial changes in adipose PI3-kinase activity or FAS. This study demonstrates that changes in carbohydrate and protein intakes within the AMDR produce fundamental shifts in glycemic regulation from high-CHO diets that require insulin-mediated peripheral glucose disposal to high-PRO diets that increase hepatic regulation of glucose appearance into the blood. J. Nutr. 136: 1855–1861, 2006.

KEY WORDS: • *insulin* • *fatty acid synthesis* • *gluconeogenesis*

Stability of blood glucose is a critical factor in lifelong health (1), yet abnormalities in glycemic control are increasingly common in adults (2) and represent a central feature of many chronic diseases including type 2 diabetes (1), the metabolic syndrome (3,4), and obesity (5). Glucose tolerance and insulin sensitivity can be improved with increased physical activity and reductions in body fat. Similarly, evidence is accumulating that diets with reduced carbohydrate:protein (CHO:PRO) ratios improve glycemic control (6–12).

Blood glucose is maintained within a narrow physiologic range through a complex balance of dietary intake, de novo synthesis, glycogen storage and release, and insulin-dependent and noninsulin-dependent glucose uptake by tissues. Use of blood glucose as a fuel is relatively constant for tissues including brain, nervous system, RBC, and kidney. These tissues account for obligatory glucose usage (13) and form the basis for the current recommended dietary allowances (RDA)[4] for dietary carbohydrates (14). Other tissues, most notably skeletal muscle, utilize a mixture of fuels (carbohydrates, lipids, and protein) determined by physiological conditions and substrate availability. Glucose use by skeletal muscle is increased during high-intensity exercise (15); during sedentary periods, increased carbohydrate intake suppresses fatty acid oxidation (16,17). Consumption of dietary glucose in excess of immediate energy needs requires storage as glycogen in liver or skeletal muscle or conversion into fat via endogenous fatty acid synthesis.

The dietary reference intakes (DRIs) for the macronutrients suggest an acceptable range for carbohydrates of 45–65% of energy intake with a minimum RDA of 130 g/d and for protein of 10–35% of energy intake with a minimum RDA of 0.8 g/kg (14). Although the DRIs establish acceptable macronutrient

[1] Presented in part at Experimental Biology 04, April 2004, Washington, DC [Baum JI, Layman DK. A paradigm shift in glycemic control using low carbohydrate (CHO), high protein (Pro) diets (abstract). FASEB J. 2004;18:A854].

[2] Support for this research was provided by the Illinois Council on Food and Agricultural Research (D.K.L.) and National Institutes of Health DK064862 (G.G.F.).

[3] To whom correspondence should be addressed. Email: dlayman@uiuc.edu.

[4] Abbreviations used: AMDR, acceptable macronutrient distribution range; BCAA, branched-chain amino acid; CHO:PRO, carbohydrate:protein ratio; DRI, dietary reference intakes; EAA, essential amino acid; FAS, fatty acid synthase; GNG, gluconeogenesis; I:G, insulin: glucagon ratio; PEPCK, phosphoenolpyruvate carboxylase; PI3-kinase, phosphatidylinositol 3-kinase; RDA, recommended dietary allowances.

Manuscript received 2 March 2006. Initial review completed 8 March 2006. Revision accepted 25 April 2006.

distribution ranges (AMDR), few studies have evaluated differences in metabolic regulations when AMDR are applied to diets. We proposed that within DRI acceptable intakes, there is a fundamental shift in glycemic regulation ranging from insulin-mediated peripheral glucose disposal associated with high dietary carbohydrates to increased hepatic regulation of glucose appearance into the blood and reduced insulin action associated with a lower-carbohydrate/higher-protein diet (10,18). The importance of this repartitioning of carbohydrate metabolism on long-term glucose homeostasis is unknown.

We hypothesized that rats trained to consume food as meals would mimic the hormonal regulations and glycemic controls observed in humans and would be an appropriate model for evaluating physiological regulations of blood glucose. To explore the molecular mechanisms controlling the balance of hepatic vs. peripheral regulation of blood glucose, we utilized 2 dietary patterns that differ in macronutrient ratios of carbohydrate to protein (CHO:PRO) and represent the AMDR of the DRI recommendations (14). Our goal was to evaluate metabolic outcomes associated with ratios of CHO:PRO >4.0 and <1.5.

MATERIALS AND METHODS

Experimental design. To characterize glycemic regulations associated with 2 dietary treatments, meal-fed adult rats were used to monitor metabolic changes during the transition from overnight food deprivation through the absorption period after a test meal. Rats were housed individually in a sedentary cage environment to minimize glucose use by skeletal muscle. Glycemic control was characterized with plasma measurements of glucose, insulin, glucagon, and corticosterone before and after the test meal. Liver glucose production was estimated using measurements of changes in plasma glucose specific activity, liver glycogen content, and phosphoenolpyruvate carboxylase (PEPCK) mRNA. Peripheral disposal of glucose was evaluated in skeletal muscle using phosphatidylinositol 3-kinase (PI3-kinase) activity as a marker of intracellular insulin signaling, and the rate of glucose uptake and muscle glycogen content as metabolic outcomes of glucose disposal. In adipose tissue, measurements included PI3-kinase activity to represent insulin signaling and glucose uptake and fatty acid synthase (FAS) mRNA as indices of glucose disposal.

Animals and diets. Male Sprague-Dawley rats (184 ± 1.0 g; Harlan-Teklad) were maintained at 24°C with a 12-h light:dark cycle and free access to water. Rats were fed diets equal in energy with macronutrient contents of 60% carbohydrate, 12% protein, 28% lipids (n = 30; CHO) or 42% carbohydrate, 30% protein, 28% lipids (n = 30; PRO; **Table 1**). The meal pattern consisted of a small (3-g) first meal of the day consumed between 0800 and 0815 followed by free access to diets from 1300 to 1400 and 1800 to 1900, representing a meal pattern designed to mimic human eating behavior (19). After 25 d of feeding, rats were examined at 3 points: before the first meal (0800) and 30 min or 90 min after the meal. At the specific times, rats were killed and blood and tissue samples collected. Gastrocnemius, soleus, and plantaris muscles, epididymal fat pads and liver were excised and immediately frozen in liquid nitrogen for later analysis.

TABLE 1

Diet composition of the CHO and PRO diets

Component	CHO group		PRO group	
	g/kg	*% energy*	*g/kg*	*% energy*
Casein	116.7	11.8	313.4	29.7
Cornstarch	400.6	37.8	280.4	26.6
L-Cystine	1.8		4.7	
Maltodextrin	134.1	12.7	93.9	8.9
Sucrose	101.5	9.6	71	6.7
Soybean oil	131.9	28.1	131.9	28.1
Cellulose (fiber)	53.7		53.7	
Mineral mix[1]	37.6		37.6	
Vitamin mix[1]	10.7		10.7	
Choline bitartrate	2.7		2.7	

[1] AIN-93 mineral and vitamin supplements (33) from Harlan Teklad.

Plasma measurements. Plasma glucose was measured using a glucose oxidase kit (ThermoTrace). Commercial RIA kits were used to measure plasma insulin and glucagon (Linco Research) and plasma corticosterone (Diagnostic Products). Free fatty acids (FFA) were measured with an enzymatic colorimetric kit (Wako Chemicals). Plasma amino acids were analyzed by ion exchange with ninhydrin detection using a Beckman 6300 amino acid analyzer.

Glucose uptake. Plantaris muscles (~200 mg) and epididymal fat (~300 g) were used for determination of glucose uptake. Rats (n = 10/diet treatment) were administered an i.p. injection of 0.5 μCi 2-deoxy-D-[2,6-^{3}H]-glucose (Amersham Biosciences) 60 min before blood was collected. Measurements were made for time 0 (pre-meal) and 90 min (postmeal). The rate of glucose uptake was determined using intracellular concentrations of 2-deoxy-D-[2,6-^{3}H]-glucose divided by plasma specific activity divided by 60 min for the incorporation time (20). Relative change in glucose uptake was determined as the percentage of change from time 0 (fasted) to 90 min after the test meal.

Muscle and liver glycogen content. Muscle and liver glycogen content were measured by phenol-sulfuric acid colorimetric assay (21).

PI3-kinase activity. Gastrocnemius muscles and epididymal fat pads were used to determine insulin receptor substrate 1–associated PI3-kinase activity as previously described (22).

Determination of PEPCK. Hepatic PEPCK mRNA content was determined by RT-PCR. Trizol, oligo(deoxythymidine) primers, SuperScript III reverse transcriptase, *Taq* DNA polymerase, and cDNA primers were purchased from Invitrogen. Total RNA was extracted from ~100 mg frozen tissue using Trizol (Invitrogen) and first-strand cDNAs synthesized using SuperScript III reverse transcriptase and oligo(deoxythymidine) primers. The reverse transcription product (5 μL) was amplified by PCR using *Taq* DNA polymerase and PEPCK specific primers (sense: 5′-AGCCTCGACAGCCTGCCCCAGG-3′, antisense: 5′-CCAGTTGTTGACCAAAGGCTTTT-3′) with 18S ribosomal RNA as an internal control. The RT-PCR product was analyzed via densitometry (Kodak Image Software).

Determination of FAS. Adipose and liver FAS mRNA was determined by real-time RT-PCR (23). Briefly, total RNA was extracted from ~100 mg frozen adipose tissue and ~50 mg frozen liver tissue by the Trizol method. First-strand cDNAs were synthesized using random hexamers. Quantitative RT-PCR with SYBR Green fluorescence dye (Applied Biosystems) was performed with FAS-specific primers (sense: 5′-TCGGCGAGTCTATGCCACTATT-3′, antisense: 5′-ACAGAGAACGGATGAGTTGT TCCT-3′) with 18S ribosomal RNA as an internal control. Primers were purchased from MWG Biotech.

Statistical analysis. Results are expressed as means ± SEM. Significance between treatment groups for all measurements was determined by 2-way ANOVA (SAS Institute) unless stated otherwise. If a significant effect was found, subsequent post hoc analyses were done using Student's t test. Differences with P-values < 0.05 were considered significant. Plasma glucose, specific activity, glycogen levels, plasma FFA, and PEPCK and FAS expression were evaluated using 2 × 3 repeated-measures ANOVA with the main effects of time and diet × time. Plasma hormones and PI3-kinase activity were evaluated as between-diet differences at each time point.

RESULTS

Food intake and body weight. Daily food intake and daily weight gain did not differ between rats administered the 2 dietary treatments (**Table 2**). However, the PRO group had 3% higher final body weights ($P < 0.05$) than the CHO group. In addition, the PRO group had greater soleus, plantaris, and

TABLE 2

Food intake and body weight for rats after consuming a CHO or PRO diet for 24 d[1]

	CHO group	PRO Group
	g	
Initial body weight	184 ± 1.0	184 ± 1.0
Final body weight	313 ± 3.2	324 ± 2.9*
Daily food intake[2]	14.1 ± 0.4	13.5 ± 0.3
Daily weight gain[2]	5.4 ± 0.6	6.1 ± 0.7
Tissue weight		
Muscle		
Soleus	0.119 ± 0.002	0.127 ± 0.003*
Plantaris	0.350 ± 0.005	0.361 ± 0.005*
Gastrocnemius	1.669 ± 0.055	1.767 ± 0.032*
Fat pad		
Epididymal	1.801 ± 0.075	1.897 ± 0.063
Renal	0.787 ± 0.040	0.736 ± 0.032

[1] Values represent means ± SEM, $n = 30$. *Different from CHO, $P < 0.05$.
[2] Daily food intake and body weight gain represent means for d 5 through 25.

gastrocnemius muscle weights than the CHO group ($P < 0.05$), whereas adipose tissue weights did not differ between the groups.

Glucose, fatty acids, and amino acids. Premeal plasma glucose was 10% higher in the PRO group compared with the CHO group ($P < 0.05$; **Table 3**). In response to the test meals, plasma glucose increased in the CHO group by 26% ($P < 0.05$) with no change in the PRO group ($P > 0.1$), producing a higher blood glucose concentration ($P < 0.05$) in the CHO group 90 min after the meal. These results are similar to blood glucose concentrations in humans consuming diets with the same macronutrient ratios (10).

After overnight food deprivation, liver glycogen concentration was lower in the CHO group than in the PRO group ($P < 0.05$; Table 3). Liver glycogen increased in the CHO group at 30 and 90 min after the meal, but did not change in the PRO group. These data indicate that the PRO group had minimal changes in liver glycogen during short-term food deprivation or postmeal periods, whereas the CHO group utilized liver glycogen to maintain blood glucose during the overnight period with recovery of liver glycogen after the meal.

The CHO group maintained the 17% higher skeletal muscle glycogen concentration ($P < 0.05$; Table 3) during the overnight period compared with the PRO group. Muscle glycogen concentrations increased for both treatment groups after the meal.

Plasma FFA concentrations were higher ($P < 0.05$) in the PRO group at 0 and 90 min compared with the CHO group (Table 3). Both treatment groups had decreased plasma FFA concentrations after the meal.

Plasma essential amino acid concentrations (Σ EAA) were higher in the PRO group after 12 h of food deprivation and after the test meal (**Table 4**). Individual amino acids followed this general pattern with branched-chain amino acid (BCAA) concentrations higher in the PRO group at both time points ($P < 0.05$). The dispensable amino acids, alanine and glutamine, were lower in the PRO group at both time points ($P < 0.05$).

Hormones. Premeal plasma insulin concentrations were lower in the CHO group ($P < 0.05$; **Fig. 1**A), consistent with the lower plasma glucose concentration in this group. After the test meals, both groups exhibited increases in plasma insulin; thus, the insulin concentrations at 30 or 90 min did not differ between the treatment groups. Although postprandial insulin concentrations were similar, the insulin response to the respective treatments differed. The CHO group had a 4-fold increase in plasma insulin levels from 0 to 30 min, whereas plasma insulin concentration doubled in the PRO group at 30 min after the meal. Further, the response to the meal was disproportionate to carbohydrate intake. The PRO group were

TABLE 3

Pre- and postmeal plasma glucose, FFA, and tissue glycogen of rats consuming a CHO or PRO diet for 24 d[1]

	Premeal[2]	Postmeal[3], *30 min*	Postmeal[3], *90 min*	Time effect	Diet × Time effect
				P-value	*P*-value
Plasma glucose, *mmol/L*					
PRO	8.89 ± 0.21	9.02 ± 0.34	9.32 ± 0.17	0.1223	0.0001
CHO	8.03 ± 0.01	8.60 ± 0.17	10.15 ± 0.42	0.0007	
Glucose specific activity,[4] *Bq/mmol*					
PRO	111.7 ± 15.8	69.0 ± 3.68	77.8 ± 14.2	0.1546	0.0004
CHO	162.6 ± 9.41	80.0 ± 4.78	70.5 ± 3.02	0.0007	
Glycogen, *mg/g tissue*					
Liver					
PRO	8.02 ± 0.49	7.35 ± 0.71	7.33 ± 0.55	0.3633	0.0135
CHO	6.11 ± 0.61	9.56 ± 0.96	8.66 ± 0.61	0.0087	
Muscle					
PRO	1.02 ± 0.06	1.55 ± 0.12	1.38 ± 0.07	0.0013	0.0001
CHO	1.23 ± 0.09	1.68 ± 0.08	1.68 ± 0.06	0.0015	
Plasma FFA, *mmol/L*					
PRO	2.17 ± 0.06	1.84 ± 0.04	1.94 ± 0.05	0.0116	NS[5]
CHO	2.04 ± 0.04	1.83 ± 0.05	1.79 ± 0.05	0.0021	

[1] Values are means ± SEM, $n = 10$.
[2] After 12 h of food deprivation.
[3] Samples collected 30 and 90 min after rats consumed the 3-g test meal.
[4] Plasma glucose specific activity 60 min after i.p. injection of 2-deoxy-[2,6-^{3}H]-glucose.
[5] NS, not significant.

TABLE 4

Plasma amino acid concentrations before and after a test meal in rats consuming a CHO or PRO diet for 24 d[1]

	PRO group		CHO group	
	Premeal[2]	Postmeal[3]	Premeal[2]	Postmeal[3]
		μmol/L		
Leucine	141 ± 2[b]	174 ± 4[a]	96 ± 3[d]	116 ± 3[c]
Isoleucine	87 ± 8[a]	94 ± 2[a]	58 ± 2[b]	64 ± 1[b]
Valine	164 ± 15[b]	218 ± 25[a]	111 ± 5[c]	151 ± 17[b]
Phenylalanine	61 ± 4[bc]	71 ± 1[a]	57 ± 1[c]	65 ± 3[b]
Threonine	388 ± 9[b]	460 ± 35[a]	319 ± 12[c]	341 ± 16[c]
Alanine	398 ± 17[d]	551 ± 11[b]	434 ± 6[c]	666 ± 23[a]
Glutamine	819 ± 30[b]	718 ± 46[c]	913 ± 28[a]	863 ± 51[b]
Σ BCAA	392 ± 13[a]	487 ± 20[a]	264 ± 8[c]	332 ± 4[b]
Σ EAA[4]	1712 ± 40[b]	1921 ± 42[a]	1435 ± 136[c]	1579 ± 46[c]
Σ Ala + Gln	1217 ± 95[b]	1268 ± 43[b]	1347 ± 108[ab]	1528 ± 51[a]

[1] Values are means ± SEM, $n = 6$. Means in a row without a common superscript letter differ, $P < 0.05$.
[2] After 12 h of food deprivation.
[3] Samples collected 90 min after rats consumed the 3-g test meal.
[4] Σ EAA = Σ BCAA + Phe + Thr + Lys + Trp + His + Met.

fed 1.3 g of carbohydrate in the 3-g test meal, and postmeal blood insulin increased 206 pmol/L (i.e., 158 pmol/L/g dietary carbohydrate), whereas the CHO group consumed 1.8 g of carbohydrates and plasma insulin levels increased by 392 pmol/L (218 pmol/L/g carbohydrate).

Premeal plasma glucagon concentrations were lower in the CHO group than in the PRO group ($P < 0.05$). After the test meal, the PRO group exhibited a glucagon response at 30 min that remained elevated at 90 min compared with the CHO group. The CHO group had no glucagon response to the meal (Fig. 1*B*).

The insulin:glucagon (I:G) ratio had no treatment × time changes ($P > 0.1$) for the PRO group during the transition from premeal to postprandial periods, whereas the CHO group had large postprandial changes in the I:G ratio (Fig. 1C).

Premeal plasma corticosterone concentrations did not differ between the 2 dietary treatments (Fig. 1*D*). The postprandial corticosterone concentrations tended to increase at 30 min ($P < 0.078$); however, the corticosterone concentration decreased ($P < 0.05$) for the CHO group at 90 min. In examining the overall hormone changes, the CHO group exhibited large insulin responses to the meal with reductions in corticosterone and stable glucagon, whereas the PRO group exhibited minimal changes in insulin and the I:G ratio with a trend toward increasing corticosterone after the meal.

PI3-kinase activity. PI3-kinase activity in skeletal muscle was lower in the CHO group after food deprivation compared with the PRO group (**Fig. 2***A*). Similar to changes in insulin and the I:G ratio, the CHO group had increased PI3-kinase activity after the meal that returned to premeal levels by 90 min. The PRO group had no significant postmeal changes in PI3-kinase activity.

Adipose PI3-kinase levels were not different in the PRO and CHO treatment groups after overnight food deprivation (Fig. 2*B*). Similar to muscle PI3-kinase activity, the PRO group had minimal change in adipose PI3-kinase levels in response to the meal. However, adipose PI3-kinase activity increased by >50% in the CHO group at 30 min and 100% at 90 min after the test meal. Adipose PI3-kinase activity was significantly greater for the CHO group than the PRO group at both 30 and 90 min.

Glycemic regulations. Differences in plasma glucose specific activities (Table 3) reflect dilution of the injected dose of ^{3}H-deoxyglucose by differential rates of glucose appearance from the liver. After overnight food deprivation, the PRO group had lower specific activity ($P < 0.05$) compared with the CHO group. Lower specific activity reflects greater dilution of the isotope by glucose derived from either glycogen breakdown or synthesis via GNG. These data suggest that the PRO group maintains higher fasting blood glucose at least in part due to a higher rate of glucose release from the liver.

After the test meals, glucose specific activity declined ~60% in the CHO group and 30% in the PRO group, reflecting the increased appearance of nonlabeled glucose from the meal. Specific activities did not differ between treatment groups at 90 min. These results demonstrate a diet × time interaction, with the CHO group exhibiting a disproportion response to the meal carbohydrate load.

Capacity for liver gluconeogenesis (GNG) was estimated as the expression of PEPCK mRNA. After 12 h of food deprivation, PEPCK expression was higher in livers of the PRO group compared with the CHO group ($P < 0.05$). After the test meal, PEPCK mRNA abundance did not change in the PRO group, whereas PEPCK mRNA decreased in the CHO group. At 90 min after the test meal, PEPCK expression in the CHO group was less than one-half that of the PRO group ($P < 0.05$; **Table 5**).

Glucose uptake into muscle and adipose tissue was measured to evaluate changes in peripheral glucose disposal and a physiologic outcome associated with insulin and PI3-kinase activity. Before the test meals, glucose uptake in skeletal muscle did not differ between the PRO and CHO groups [88.9 ± 31.1 vs 53.1 ± 12.3 nmol/(g tissue·min), $P > 0.1$]. After the meals, both treatment groups increased glucose uptake into skeletal muscle. However, the increase in glucose uptake was greater for the CHO group (**Fig. 3**). The glucose uptake is consistent with greater changes in insulin concentration and PI3-kinase activity in this group. Although glucose uptake into muscle was greater for the CHO group, the relative change in tissue glycogen concentration was similar for the PRO and CHO groups (Table 3).

In adipose tissue, glucose uptake was greater in the PRO group before the meal [33.8 ± 9.2 vs 15.0 ± 3.3 nmol/(g tissue·min), $P < 0.05$]. After the meal, the CHO group had a significant increase in glucose uptake 90 min after the meal, consistent with changes in PI3-kinase activation. The change in glucose uptake after the meal in the CHO group was ~3 times that in the PRO group (Fig. 3).

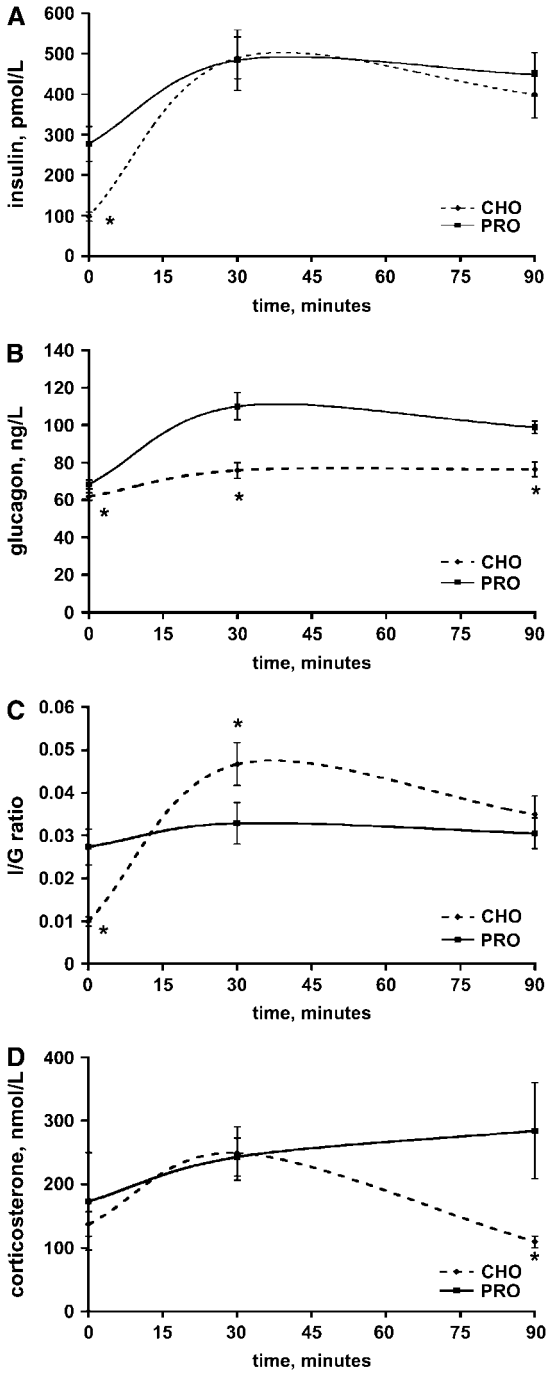

FIGURE 1 Plasma insulin (*A*) and glucagon (*B*) concentrations, the I:G glucagon ratio (*C*), and the corticosterone (*D*) concentration in rats consuming CHO or PRO diets for 24 d before and 30 and 90 min after a test meal. Values are means ± SEM, $n = 10$. *Different from PRO at that time, $P < 0.05$.

Fatty acid synthase (FAS). Changes in hepatic and peripheral fatty acid synthesis were estimated as FAS mRNA abundance. Liver FAS did not differ between the groups, but adipose FAS was higher in the CHO group (Table 5).

DISCUSSION

The stability of blood glucose requires balance between hepatic glucose release and peripheral glucose disposal. The liver regulates the rate of glucose appearance into the blood by balancing absorption of exogenous dietary glucose with endogenous production of glucose from GNG and glycogen breakdown (24–27). The use of blood glucose by peripheral tissues occurs through insulin-dependent and insulin-independent transporters (28,29). The goal of this study was to evaluate differences in these metabolic regulations associated with macronutrient CHO:PRO ratios representing the AMDR of the DRIs. Results from this study support the hypothesis that dietary macronutrient changes within the DRI guidelines produce fundamental shifts in glycemic regulation from high-carbohydrate diets that rely on insulin-mediated peripheral glucose disposal to reduced-carbohydrate diets that rely to a greater extent on hepatic regulation of glucose appearance into the blood.

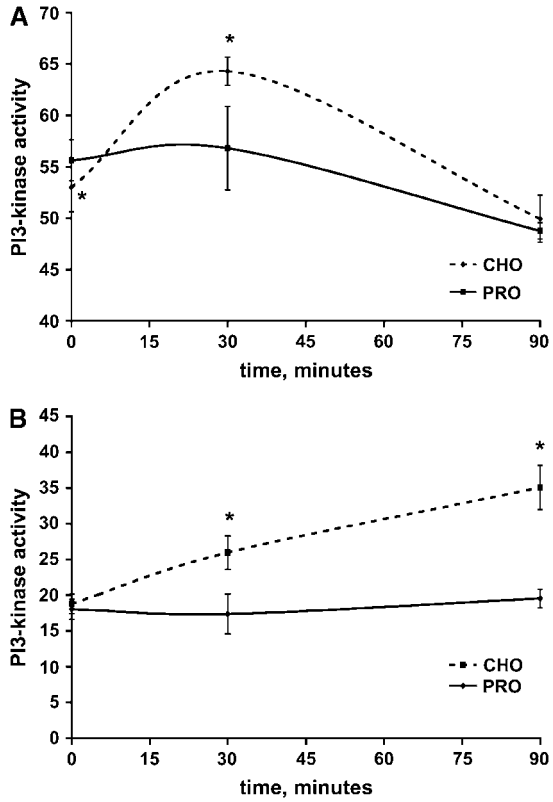

FIGURE 2 Skeletal muscle (*A*) and adipose tissue (*B*) PI3-kinase activity in rats consuming CHO or PRO diets for 24 d before and 30 and 90 min after a test meal. Values are means ± SEM (AU), $n = 5$. *Different from PRO at that time, $P < 0.05$.

The 2 treatment groups were each fed 3 meals/d with diets equal in total energy, lipids, and fiber. Diets differed only in the CHO:PRO ratios. The CHO group consumed a diet with energy provided as 60% CHO, 12% PRO, and 28% lipids, whereas the PRO group consumed a diet with 42% CHO, 30% PRO, and 28% lipids. The meal protocol was used to mimic the chronic fed and fasting metabolic patterns of human diets.

Comparing the 2 groups at 0800 before the first meal, the CHO group had lower plasma glucose concentration and higher glucose specific activity. The higher specific activity suggests a lower rate of release of unlabeled glucose from the liver. Supporting the findings of lower hepatic glucose release, the CHO group had reduced liver glycogen concentration, reflecting depletion of glycogen stores and lower expression of hepatic PEPCK consistent with downregulation of GNG. The CHO group also had reduced levels of plasma insulin and reduced glucose uptake into adipose tissue and skeletal muscle,

TABLE 5

Liver PEPCK mRNA and liver and adipose FAS mRNA of rats consuming a CHO or PRO diet for 24 d[1,2]

	Premeal[3]	Postmeal[4], *30 min*	Postmeal[4], *90 min*	Time effect	Diet × Time effect
				P-value	*P*-value
PEPCK		AU			
Liver					
PRO	17.0 ± 1.7	20.0 ± 3.1	15.7 ± 2.2	0.6619	0.0072
CHO	12.2 ± 1.9	8.6 ± 3.1	7.1 ± 1.3	0.0685	
FAS					
Liver					
PRO	65.6 ± 15.1	165.5 ± 67.3	190.8 ± 90.5	0.1628	0.1561
CHO	46.5 ± 33.6	92.4 ± 24.3	142.6 ± 73.1	0.0821	
Adipose					
PRO	12.4 ± 2.2	26.8 ± 10.5	27.1 ± 13.4	0.8198	0.0129
CHO	25.8 ± 6.7	59.6 ± 27.0	38.1 ± 8.1	0.3936	

[1] Values are means ± SEM, $n = 5$.
[2] mRNA was normalized to 18S RNA.
[3] After 12 h of food deprivation.
[4] Samples collected 30 and 90 min after rats consumed a 3-g test meal.

further supporting a lower rate of glucose appearance into the blood.

Contrary to this metabolic pattern, the PRO group had higher plasma glucose concentration, lower glucose specific activity (i.e., increased glucose release), higher liver glycogen concentration, and increased levels of PEPCK mRNA before the test meal. These findings are consistent with increased hepatic glucose production. The PRO group also had increased plasma insulin concentration and increased glucose uptake into adipose and skeletal muscle during the period of food deprivation.

The current study is in agreement with the findings of Rossetti et al. (30), who reported that rats fed a diet containing 60% CHO, 18% PRO, and 22% lipids had lower fasting blood glucose and insulin concentrations followed by higher postprandial glucose and insulin concentrations than rats fed a low-carbohydrate, higher-protein diet (43% CHO, 35% PRO, 22% lipids). These investigators also measured glucose flux and found that rats that consumed the low-protein diet had a reduced rate of hepatic glucose production compared with those fed the higher-protein diet. Further, Peret et al. (31) reported that PEPCK activity decreases as the concentration of carbohydrates in the diet increases and protein decreases.

After the test meal, rats in the CHO group increased plasma glucose and insulin and liver glycogen concentration. Skeletal muscle responded to the meal with a rapid increase in PI3-kinase signaling activity accompanied by increased glucose uptake and increased muscle glycogen. Adipose tissue responded to the meal by increasing PI3-kinase activity; however the pattern of increase was unlike that in skeletal muscle. Muscle PI3-kinase activity changed in parallel with changes in plasma insulin concentrations with a peak at ~30 min after the meal, whereas adipose PI3-kinase activity exhibited a delayed response with activity still increasing at 90 min after the meal. Associated with increased PI3-kinase activity, adipose tissue had a 5-fold increase in glucose uptake. Adipose tissue also exhibited a significant increase in the expression of FAS in rats fed the high-CHO diet. Taken together, these data suggest that adipose tissue disposes of dietary carbohydrates that exceed the amount used by skeletal muscle.

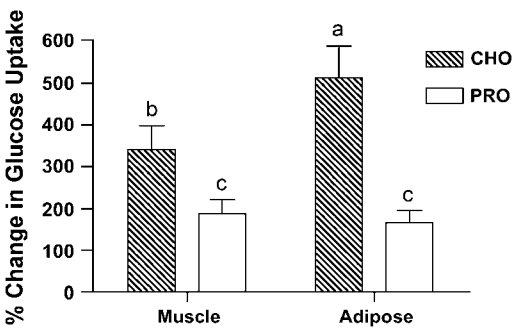

FIGURE 3 Change in uptake of 2-deoxy-D-[2,6-^{3}H]-glucose from premeal to 90 min after the test meal in skeletal muscle and adipose tissue in rats consuming CHO or PRO diets for 24 d. Values are means ± SEM, $n = 5$. *Different from PRO, $P < 0.05$.

Similarly, Kabir et al. (32) found that feeding rats a high glycemic index starch diet resulted in lower hepatic PEPCK mRNA and increased FAS mRNA in both normal and diabetic rats compared with those fed a low glycemic index diet. These investigators proposed that the postprandial hyperinsulinemia associated with a high-CHO diet reduces the expression of PEPCK and stimulates FAS activity and lipogenesis.

The PRO group produced a more limited glycemic response to the test meal. Plasma insulin concentration increased after the meal but the magnitude was less than one-half of the change in the CHO group, and blood glucose concentration returned to premeal values by 90 min after the meal. Associated with the reduced insulin response, the I:G ratio did not change in the PRO group from the overnight fast through the postprandial period. Consistent with a reduced postprandial insulin response, skeletal muscle had minimal change in PI3-kinase activity. However, glucose uptake into muscle increased after the meal, and there was a postprandial increase in muscle glycogen content similar to that in the CHO group.

The reduced peripheral insulin response of the PRO group to the test meal is explained in part by the lower carbohydrate content of the meal. However the magnitude of the metabolic response appears to be disproportionate to the differences in dietary carbohydrates. Specifically, the test meal for the CHO group contained ~40% more carbohydrates (1.8 vs. 1.3 g), whereas the insulin response at 30 min was >2-fold higher, and PI3-kinase activity in muscle and adipose tissue was unchanged

from the premeal baseline with the lower CHO test meal. In total, rats in the PRO group maintained glycemic control with a greater emphasis on hepatic regulations and less emphasis on postprandial insulin response to dispose of dietary glucose.

Plasma BCAA were higher in the PRO group before the test meal; however, the PRO group had lower concentrations of alanine and glutamine, which are produced largely in skeletal muscle associated with degradation of the BCAA and serve as substrates for GNG (18). Because of the lower protein intake in the CHO group, higher concentrations of alanine and glutamine appear inconsistent. However, an alternative explanation is the downregulation of GNG noted in this study (i.e., reduced PEPCK expression) and by others (30,31) may reduce clearance of alanine and glutamine. These findings are similar to previous reports from human weight loss studies (10,18).

This study demonstrates dramatic differences in metabolic regulations and outcomes associated with the DRI AMDR (14). In the present study with ratios of carbohydrates:protein at 5.0 or 1.4, both treatment groups were able to maintain normal glycemic control based on observed values for glucose and insulin. However, the high-CHO diet group required a highly responsive endocrine system to modulate the large swings in glucose metabolism from the food-deprived to fed periods. This regulation results in downregulation of endogenous glucose synthesis via GNG with increased emphasis on peripheral disposal of dietary carbohydrates in skeletal muscle and adipose tissue. Rats consuming a reduced CHO diet with increased protein reflect minimal diurnal variations in blood glucose or endocrine regulations, emphasize endogenous glucose production via GNG, and minimize the need for postprandial glucose clearance by adipose tissue. The long-term implications of these dietary patterns for glycemic control and adult health warrant evaluation.

LITERATURE CITED

1. DeFronzo RA, Bonadonna RC, Ferrannini E. Pathogenesis of NIDDM. A balanced overview. Diabetes Care. 1992;15:318–68.
2. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults. JAMA. 2002;287:356–9.
3. Reaven GM. Role of insulin resistance in human disease (syndrome X): an expanded definition. Annu Rev Med. 1993;44:121–31.
4. Hansen BC. The metabolic syndrome X. Ann N Y Acad Sci. 1999;892: 1–24.
5. Klein S. Outcome success in obesity. Obes Res. 2001;9: Suppl 4:S354–8.
6. Ludwig DS. Dietary glycemic index and obesity. J Nutr. 2000;130: Suppl 2S:280S–3.
7. Layman DK. The role of leucine in weight loss diets and glucose homeostasis. J Nutr. 2003;133:261S–7.
8. Klein S, Sheard NF, Pi-Sunyer X, Daly A, Wylie-Rosett J, Kulkarmi K, Clark NG. Weight management through lifestyle modification for the prevention and management of type 2 diabetes. A statement of the American Diabetes Association, the North American Association for the Study of Obesity, and the American Society for Clinical Nutrition. Am J Clin Nutr. 2004;80:257–63.
9. Layman DK, Boileau RA, Erickson DJ, Painter JE, Shiue H, Sather C, Christou DD. A reduced ratio of dietary carbohydrate to protein improves body composition and blood lipid profiles during weight loss in adult women. J Nutr. 2003;133:411–7.
10. Layman DK, Shiue H, Sather C, Erickson DJ, Baum J. Increased dietary protein modifies glucose and insulin homeostasis in adult women during weight loss. J Nutr. 2003;133:405–10.
11. Farnsworth E, Luscombe ND, Mokes M, Wittert G, Argyiou E, Clifton PM. Effect of a high-protein, energy restricted diet on body composition, glycemic control, and lipid concentrations in overweight and obese hyperinsulinemic men and women. Am J Clin Nutr. 2003;78:31–9.
12. Gannon MC, Nuttall FQ, Saeed A, Jordan K, Hoover H. An increase in dietary protein improves the blood glucose response in persons with type 2 diabetes. Am J Clin Nutr. 2003;78:734–41.
13. Cahill GF. Starvation in man. N Engl J Med. 1970;282:668–75.
14. Institute of Medicine. Food and Nutrition Board. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. National Academy Press: Washington, DC; 2002.
15. Holloszy JO, Booth FW. Biochemical adaptations to endurance exercise in muscle. Annu Rev Physiol. 1976;38:273–91.
16. Wolfe RR. Metabolic interactions between glucose and fatty acids in humans. Am J Clin Nutr. 1998;67: Suppl 3:519S–26.
17. Hellerstein MK. No common energy currency: de novo lipogenesis as the road less traveled. Am J Clin Nutr. 2001;74:707–8.
18. Layman DK, Baum JI. Dietary protein impact on glycemic control during weight loss. J Nutr. 2004;134:968S–73.
19. Morens C, Gaudichon C, Metges CC, Fromentin G, Baglieri A, Even PC, Huneau JF, Tomé D. A high-protein meal exceeds anabolic and catabolic capacities in rats adapted to a normal protein diet. J Nutr. 2000;130:2312–21.
20. Ohshima K, Shargill NS, Chan TM, Bray GA. Adrenalectomy reverses insulin resistance in muscle from obese (ob/ob) mice. Am J Physiol. 1984;246:E193–7.
21. Lo S, Russell JC, Taylor AW. Determination of glycogen in small tissue samples. J Appl Physiol. 1970;28:234–6.
22. O'Connor JC, Freund GG. Vanadate and rapamycin synergistically enhance insulin-stimulated glucose transport. Metabolism. 2003;52:666–74.
23. Cheon Y, Nara TY, Band MR, Beever JE, Wallig MA, Nakamura MT. Induction of overlapping genes by fasting and a peroxisome proliferator in pigs: evidence of functional PPARalpha in non-proliferating species. Am J Physiol Regul Integr Comp Physiol. 2005;288:R1525–35.
24. Jahoor F, Peters EJ, Wolfe RR. The relationship between gluconeogenic substrate supply and glucose production in humans. Am J Physiol. 1990;258:E288–96.
25. Balasubramanyam A, McKay S, Nadkarni P, Rajan AS, Garza A, Pavlik V, Herd JA, Jahoor F, Reeds PJ. Ethnicity affects the postprandial regulation of glycogenolysis. Am J Physiol. 1999;277:E905–14.
26. Christiansen MP, Linfoot PA, Neese RA, Hellerstein MK. Effect of dietary energy restriction on glucose production and substrate utilization in type 2 diabetes. Diabetes. 2000;49:1691–9.
27. Pascual M, Jahoor F, Reeds PJ. Dietary glucose is extensively recycled in the splanchnic bed of fed adult mice. J Nutr. 1997;127:1480–8.
28. Furtado LM, Somwar R, Sweeney G, Niu W, Klip A. Activation of the glucose transporter GLUT4 by insulin. Biochem Cell Biol. 2002;80:569–78.
29. Wood IS, Trayhurn P. Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. Br J Nutr. 2003;89:3–9.
30. Rossetti L, Rothman DL, DeFronzo RA, Shulman GI. Effect of dietary protein on in vivo insulin action and liver glycogen repletion. Am J Physiol. 1989;257:E212–9.
31. Peret J, Chanez M, Cota J, Macaire I. Effects of quantity and quality of dietary protein and variation in certain enzyme activities on glucose metabolism in the rat. J Nutr. 1975;105:1525–34.
32. Kabir M, Rizkalla SW, Qhignard-Boulange A, Guerre-Millo M, Boillot J, Ardouin B, Luo J, Slama G. A high glycemic index starch diet affects lipid storage-related enzymes in normal and to a lesser extent in diabetic rats. J Nutr. 1998;128:1878–83.
33. Reeves PG. Components of the AIN-93 diets as improvements in the AIN-76A diet. J Nutr. 1997;127: Suppl 5:838S–41.