

**Original Article****Effects of High Protein Diets on Brush Border Membrane Enzymes and Carbohydrate Metabolism in Rat Intestine and Liver**Neelam Farooq<sup>1</sup>, Samina Salim<sup>2</sup>, Ahad Noor Khan Yusufi<sup>1,\*</sup>

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**ABSTRACT**

**Background and Objective:** We are what we eat. Adequate balanced nutrition is important to maintain health. However, sugar, fat and protein-rich diets, fried and processed foods and so called popular or TV diets such as Atkins diet cause negative effects on human health. Primarily, they affect structure and functions of the intestine, liver and kidney. In the present study, effects of high protein diets were assessed on various serum parameters and on enzymes of brush border membrane and energy yielding metabolic pathways such as glycolysis, TCA cycle, gluconeogenesis HMP shunt in small intestine and liver of rats.

**Materials and Methods:** Rats were fed with high protein diets for 28 d. At the end of the experiment, rats were sacrificed under light ether anaesthesia and blood samples were collected and small intestines and livers were extracted and processed for the preparation of homogenates and brush border membrane vesicles (BBMV). The present study was carried out to investigate the effects of HPD on body weights, serum parameters and enzymes of carbohydrate metabolism in BBM of the rats' intestines and livers.

**Results:** Results showed that high protein diets increased serum glucose and decreased inorganic phosphate; however, serum cholesterol and phospholipids were unchanged. High protein diets significantly increased the activity of alkaline phosphatase (ALkPase) and  $\gamma$ -glutamyl transferase (GGTase) in mucosal brush border membranes but decreased the sucrase activity. The activity of metabolic enzymes of lactate dehydrogenase (LDH) involved in glycolysis and malate (MDH), succinate (SDH) and isocitrate (ICDH) dehydrogenase involved in TCA cycle significantly decreased by high protein diets in the intestine. The activity of glucose-6-phosphate dehydrogenase (G6PDH, HMP shunt) and NADP-malic enzyme (ME) significantly decreased; however, gluconeogenesis enzymes of glucose-6-phosphatase (G6Pase) and fructose-6-bisphosphatase (FBPase) increased in intestine by high protein diets. High protein diets decreased the activity of LDH and MDH whereas increased the activity of FBPase, G6Pase, G6PDH and ME in the liver.

**Conclusion:** In conclusion, consumption of high protein diets caused extensive alterations in the intestinal brush-border membrane enzymes. The activity of enzymes of glycolysis and TCA cycle decreased but those of glucose production and HMP shunt increased by high protein diets in the intestine. The metabolic activity was differentially affected by high protein diets in the liver as shown by the changes in associated enzymes. The enzymes of glycolysis and TCA cycle decreased but those of gluconeogenesis and HMP shunt as well as ME significantly increased by high protein diets in the liver.

**Keywords:** High protein diets, Intestine, liver, Brush border membrane enzymes, Carbohydrate metabolism

**Highlights**

- Feeding rats with high protein diets increased their serum glucose and decreased Pi; however, cholesterol and phospholipids were not affected.
- High protein diets increased brush border membrane enzymes; ALkPase and GGTase but decreased sucrase activity.
- High protein diets increased ALkPase by increasing Vmax and GGTase by increasing Vmax and decreasing Km, whereas decreased sucrase by decreasing Vmax and Km.
- High protein diets decreased glycolysis, TCA cycle and HMP shunt but increased gluconeogenesis in the intestine as demonstrated by the enzymes.
- High protein diets decreased glycolysis and TCA cycle but increased HMP shunt and gluconeogenesis in the liver as shown by the enzymes.

## Introduction

We are what we eat! Adequate and balanced nutrition is important to maintain health. However, sugar, fat, or protein rich diet, fried and processed food and so called popular or TV diet e.g., Atkins diet cause negative effects on human health. Small intestine is the major primary site, where complex foods are digested to useful nutrients, absorbed and metabolized. Intestinal brush border membrane (BBM) that lines the epithelium is involved in digestion and absorption of nutrients because it includes a number of hydrolytic enzymes and transport carrier systems (1–4). Liver is involved directly or indirectly in the intestinal functions. It is the major site of the oxidative metabolism of food components. Nutritional stresses such as fasting, Ramadan fasting and restricted calorie intake and foods enriched in excess of carbohydrates, fats and proteins include adverse effects on the intestine and liver (3–9). The activity of BBM enzymes of disaccharidases (e.g. sucrase), hydrolases (e.g. ALkPase) and peptidases (e.g. GGTase) in the intestine is affected by the composition of diets. The diets rich in carbohydrates (HCD), fats (HFD) or proteins (HPD) cause specific alterations in the activity of BBM enzymes (10–13). Studies have been carried out on the effects of HCD and HFD on the intestine and liver; however, studies on the effects of HPD on the structure and energy yielding metabolic activity in the intestine and liver are especially limited. It has been reported that low-fat, high-casein or whey protein weight maintenance diets are more effective for weight control than those low-fat and high-carbohydrate diets are, not adversely affecting metabolic and cardiovascular risk factors (14). Raheja et al. (15) reported decreases in glucose oxidation by HPD in chick intestines. Melo et al. (16) reported that increase of dietary protein and decrease of carbohydrates and lipids decreased the glycolytic activity and induced hepatic gluconeogenesis as a strategy to provide metabolic energy from amino acids (AAs). Feeding of casein to fasted rat diets leads to increases in ALkPase activity in intestinal mucosa (17). The activity of ALkPase was shown to increase but that of sucrase decreased in rats fed an HPD (6, 12).

However, the effects of HPD on the biochemical events and the mechanism involved in the cellular response to HPD have not completely been explained, neither those participating in inflammation and energy yielding metabolic activities in the small intestine and liver. Therefore, the current study investigated effects of HPD on various serum parameters, enzymes of BBM and carbohydrate metabolism (e.g. enzymes of glycolysis, TCA cycle, gluconeogenesis and HMP shunt) in the rat intestine and liver under similar experimental conditions to avoid day-to-day variations. Results showed that HPD increased serum glucose and decreased inorganic phosphate; however, serum cholesterol and phospholipids were not affected. The activity of BBM

enzymes of ALkPase and GGTase increased but sucrase decreased by HPD. The increase/decrease of respective enzymes was due to the increase/decrease of Vmax and decrease of Km values. The HPD decreased the activity of enzymes of glycolysis and TCA cycle whereas increased the activity of gluconeogenesis in the intestine and liver differentially. However, HPD decreased the activity of G6PDH and ME in the intestine but increased them in the liver. In conclusion, HPD caused extensive alterations in the intestinal BBM and the liver, disrupted metabolic activity differentially as indicated by the changes in associated enzymes and other parameters.

## Materials and Methods

### Chemicals

Casein, *p*-nitro phenyl phosphate, NADH and NADP<sup>+</sup> were purchased from Sigma, St Louis, MO, USA. All other chemicals included analytical grade and were purchased from Sigma, St Louis, MO, USA, or Sisco Research Laboratory, Mumbai, India.

### Animals

Adult albino rats (Wistar strain) were purchased from All India Institute of Medical Sciences, New Delhi, India.

### Diets

#### Normal Control Diets

Standard rat pellet diets were purchased from Amrut, Maharashtra, India.

#### Preparation of High Protein Diets

The following ingredients were mixed with powdered NCD to form HPD crude protein content of 88% as described by Wolfram and Scharrer (18): starch, 3%; corn oil, 2%; mineral mixture, 5% and vitamin mixture, 2%.

### Experimental Design

The experiments were carried out on male Wistar rats based on the guidelines approved by institutional ethical committee and Ministry of Environment and Forests (CPCSEA), Government of India. The rats (ten rats per group), weighing 150–200 g, were conditioned for 1 w in the animal facility and fed with standard rat diet (NCD) and water *ad libitum*. Two groups of rats were used in the study. One group of rats was fed with HPDs for 28 d as described by Wolfram and Scharrer (18). The other group received NCDs and was used as control. The body weights of rats were recorded at the beginning and the completion of the experiments. Then, rats were sacrificed under light anaesthesia. Blood samples were collected and the intestine and liver extracted and processed for the preparation of homogenates and brush border membrane vesicles (BBMV) as described as follows. All the preparations and analyses of various parameters were carried out simultaneously under similar experimental conditions to avoid day-to-day variations.

### Preparation of Homogenates and Brush Border Membranes Vesicles

After the completion of the experiment, rats' intestines were extracted. The intestines were washed by flushing them with ice-cold buffered saline (1 mM Tris-HCl, 9 g l<sup>-1</sup> NaCl; pH 7.4). The livers were transferred into tris buffered saline (TBS) as described previously (3, 4, 19). The intestinal BBMVs were prepared as described by Farooq et al. (3, 4) via CaCl<sub>2</sub> precipitation using differential centrifugation technique. Mucosa scraped from 4–5 time washed intestines was used for BBMV preparation. Briefly, the mucosal scrapings were collected using beakers containing 50 mM mannitol and 5 mM tris-HCl (pH 7.5). The mucosal homogenate was diluted with the tris-mannitol buffer (15 ml g<sup>-1</sup> tissue) and further homogenized using Ultra-Turrex T25 homogenizer (Janke and Kunkel, Staufen, Germany) with three pulses of 30 s, each with 30 s intervals. Aliquots of mucosal homogenate were prepared and quickly frozen for further analysis. Moreover, CaCl<sub>2</sub> was added to the filtrate to a final concentration of 10 mM and then stored on ice for 20 min with intermittent stirring. The homogenate was centrifuged at 2000 g for 10 min using Beckman J2-M1 refrigerated centrifuge (Beckman, Palo Alto, CA, USA) with a JA-17 rotor. The pellet was discarded and the supernatant was recentrifuged at 35000 g for 30 min. The pellet was resuspended in a small volume (1–2 ml) of 50 mM sodium maleate buffer, pH 6.8, with four complete passes using loose fitting Dounce homogenizer (Wheaton, USA) and centrifuged at 35,000 g for 30 min using 15-ml glass tubes. The white outer fluffy portion of the pellet was resuspended carefully in a small volume of the highlighted buffer. The BBM suspension was quickly frozen in small aliquots for the enzyme analyses. All the steps were strictly carried out at 0–4 °C unless otherwise specified. A 10% liver homogenate was similarly prepared in 10 mM tris-HCl buffer, pH 7.5. The homogenates were centrifuged at 2000 g for 10 min at 4 °C to remove cell debris and the supernatant was aliquoted and stored at -20 °C for assaying the enzymes of carbohydrate metabolism as previously described (19).

### Serum Chemistries

Serum samples were deproteinized with 3% trichloroacetic acid in a ratio of 1:3, set for 10 min and then centrifuged at 2000 g for 10 min. The protein free supernatant was used to assess inorganic phosphate and creatinine. Blood urea nitrogen (BUN) and cholesterol levels were assessed directly in serum samples. Glucose was estimated using *o*-toluidine method and commercial kits from Span diagnostics, Mumbai, India. These parameters were assessed via standard procedures as described in a previous study (19).

### Enzyme Assays

The activities of BBM biomarkers enzymes, alkaline phosphatase (ALPase),  $\gamma$ -glutamyl transferase (GGTase) and sucrase in the homogenates and BBM preparations were

assessed as described previously (3, 4). The enzymes of carbohydrate metabolism such as lactate (LDH), malate (MDH), glucose-6-phosphate (G6PDH) dehydrogenases and NADP-malic enzyme (ME) involved in oxidation of NADH or decrease of NADP were assessed by investigating changes at 340 nm using spectrophotometer (Cintra 5; GBC Scientific Equipment, Victoria, Australia) as described previously (3, 19). Other enzymes, including glucose-6-phosphatase (G6Pase) and fructose-1, 6-bisphosphatase (FBPase), were assessed as described in previous studies (19). Protein concentration was assessed using a modified method of Lowry et al. (20) by Yusufi et al. (21).

### Statistical Analyses

All data were expressed as mean  $\pm$  SE (standard error) for at least 4–5 various preparations. Independent samples t-test and one-way ANOVA test were used to analyze differences in the means. Level of significance was set at 5%. Most of the changes between various groups were compared to control values for better understanding and clarity. All statistical analyses were carried out using SPSS software v.20 (IBM, USA).

## Results

The present study was carried out to investigate the effects of HPD on body weights, serum parameters and enzymes of carbohydrate metabolism in BBM of the rats' intestines and livers. In general, the rats were active and alert throughout the study.

### Body Weight and Weight of Mucosa

Effects of HPD were observed on the body and intestinal mucosa weights of the rats. As shown in Table 1, feeding of HPD for 28 d caused significant increases (+42%) in the body weight of the rats, compared to control (NCD-fed) rats. The weight of intestinal mucosa was not changed significantly.

**Table 1.** Effects of high protein diets on body and intestinal mucosa weights of the rats

Groups	Body weight (g)	Mucosa weight (g)
Control	233.39 $\pm$ 2.72	12.23 $\pm$ 0.07
HPD	330.96 $\pm$ 8.86* (+42%)	12.80 $\pm$ 0.62 (+5%)

Results are mean  $\pm$  SEM of eight different preparations. Values in parenthesis represent percent change from control. \*Significantly different from corresponding control values at  $p < 0.05$  or higher degree of significance by independent *t* test and ANOVA. HPD, high protein diet

### Effects of High Protein Diets on Serum Parameters

The effects of HPD were observed on various serum parameters (Table 2). Feeding of HPD included no significant effects on serum creatinine, compared to control rats, indicating normal functioning of the kidneys. Feeding of HPD resulted in significant increases in serum glucose (+66%) and significant decreases in serum Pi (-30%); however, serum cholesterol and phospholipids were not affected by HPD.

### Effects of High Protein Diets on Brush Border Membrane Marker Enzymes in Mucosal Homogenates and Brush Border Membranes Vesicles

The effects of HPD were assessed on the activities of ALkPase, GGTase and sucrase in the homogenates and BBMV isolated from rats' intestinal mucosa (Table 3). The activities of GGTase (+19%) significantly increased whereas the activity of ALkPase (-2%) was not altered in the mucosal homogenate by HPD. In contrast, the activity of sucrase decreased significantly (-37%) by HPD in intestinal homogenates. The effects of HPD were further analyzed on the specific activities of BBM marker enzymes in BBMV isolated from intestinal mucosa. The activities of ALkPase

(+101%) and GGTase (+52%) greatly increased while the activity of sucrase (-32%) significantly decreased in the isolated BBMV of HPD fed rats (Table 3). The kinetic parameters (Vmax and Km) were assessed by assaying the enzymes in BBMV isolated from intestinal mucosa (Table 4). Results indicated that increases in ALkPase activity by HPD was due to increases in the Vmax (+20%) with a small decrease in the Km values. The GGTase activity in HPD-fed rats increased due to great increases in Vmax (+73%) with large decreases (-65%) in Km values. In contrast, the decrease in sucrase activity by HPD was due to decreases in Vmax (-33%) and Km (-59%) values (Table 4).

**Table 2.** Effects of high protein diets on various serum parameters

Parameters Groups	Creatinine (mg/dl)	Glucose (mg/dl)	Cholesterol (mg/dl)	Phospholipids (μg/ml)	Phosphate (μmol/ml)
Control	21.27 ± 0.21	66.4 ± 4.6	3.11 ± 0.01	340 ± 12	1.93 ± 0.05
HPD	20.20 ± 0.14 (-5%)	110 ± 5.4* (+66%)	3.08 ± 0.05* (-0.1%)	322 ± 8 (-5%)	1.35 ± 0.04* (-30%)

Results are mean ± SEM of eight different preparations. Values in parenthesis represent percent change from control. Significantly different from corresponding control values at p<0.05 or higher degree of significance by independent *t* test and ANOVA. HPD, high protein diet;

**Table 3.** Effects of high protein diets on the specific activities of ALkPase, GGTase and sucrase in (a) homogenate and (b) brush border membrane vesicles of the rats' intestines

Enzymes/ Groups	ALkPase <sup>1</sup> (μmol/mgprotein/h)	GGTase (μmol/mgprotein/h)	Sucrase (μmol/mgprotein/h)
(a) Homogenate			
Control	4.0 ± 0.09	1.73 ± 0.03	24.02 ± 0.47
HPD	3.95 ± 0.17 (-2%)	2.06 ± 0.023 (+19%)	15.06 ± 0.87 (-37%)
(b) BBMV			
Control	39.17 ± 1.85	15.6 ± 1.30	205.0 ± 11.85
HPD	78.58 ± 5.49* (+101%)	23.27 ± .90 (+52%)	141.3 ± 25.64* (-32%)

Results are mean ± SEM of five different preparations. Values in parenthesis represent percent change from control. \*Significantly different from corresponding control at p<0.05 or higher degree of significance by independent *t* test and ANOVA.

<sup>1</sup>ALkPase, alkaline phosphatase; GGTase, γ-glutamyl transferase; HPD, high protein diet;

**Table 4.** Effects of high protein diets on kinetic parameters of (a) ALkPase (b) GGTase and (c) sucrase in brush border membrane vesicles of the rats' small intestines

Groups/Enzymes	Vmax (μmol/mg protein/h)	K <sub>m</sub> × 10 <sup>-3</sup> M
ALkPase <sup>1</sup> (a)		
Control	18.51	0.92
HPD	22.22 (+20%)	0.83 (-8%)
(b) GGTase		
Control	5.78	1.30
HPD	10.00 (+73 %)	0.45 (-65%)
(c) Sucrase		
Control	250.00	41.66
HCD	166.66 (-33%)	17.24 (-59%)

Results are mean of five different preparations. Values in parenthesis represent percent change from control. Km (Michaelis Menton constant) and Vmax (maximal velocity of enzyme reaction).

<sup>1</sup>ALkPase, alkaline phosphatase; GGTase, γ-glutamyl transferase; HPD, high protein diet;



## Effects of High Protein Diets on the Enzymes of Carbohydrate Metabolism in Intestinal and Liver Homogenates

The effects of nutritional stress by feeding HPD were assessed on the enzymes of carbohydrate metabolic pathways such as glycolysis, TCA cycle, HMP shunt and gluconeogenesis in the rats' intestines and livers (Table 5). Feeding of HPD greatly decreased the activity of LDH (-42%) as a representative enzyme of glycolysis. In addition, activity of TCA cycle enzymes of MDH (-20%), SDH (-39%) and ICDH (-16%) decreased by HPD, compared to control rats (Table 5). The activities of enzymes involved in glucose production by gluconeogenesis, including G6Pase (+12%) and FBPase (+15%), slightly increased in HPD fed rats. The effects of HPD were assessed on the activities of

glucose-6-phosphate dehydrogenase (G6PDH) and ME in mucosal homogenates that provided NADPH needed in various anabolic reactions and antioxidant mechanisms. Feeding of HPD to the rats significantly decreased the activity of G6PDH (-17%). However, the activity of ME (-76%) greatly decreased in the intestine by HPD (Table 5). The activity of enzymes involved in metabolic pathways was compared with that of liver under the highlighted diet. Similar to intestine, HPD caused great decreases in the activity of LDH (-36%) and MDH (-32%) in liver homogenates. Unlike intestine, the activity of FBPase (+40%) and G6Pase (+27%) was greatly enhanced by HPD in the liver. Furthermore, HPD increased the activity of G6PDH (+42%) and ME (+22%) in the liver (Table 5).

**Table 5.** Effects of high protein diets on the specific activities of carbohydrate metabolic enzymes in homogenates of the rats' intestines and livers

Enzymes/ Groups	LDH <sup>1</sup> ( $\mu\text{mol}/\text{mg}$ protein/h)	MDH ( $\mu\text{mol}/\text{mg}$ protein/h)	FBPase ( $\mu\text{mol}/\text{mg}$ protein/h)	G6Pase ( $\mu\text{mol}/\text{mg}$ protein/h)	G6PDH ( $\text{nmol}/\text{mg}$ protein/h)	ME ( $\text{nmol}/\text{mg}$ protein/h)	SDH ( $\text{nmol}/\text{mg}$ protein/h)	ICDH ( $\text{nmol}/\text{mg}$ protein/h)
<b>Intestine</b>								
Control	52.0 $\pm$ 5.6	49.2 $\pm$ 9.3	1.46 $\pm$ 0.05	4.17 $\pm$ 1.0	213 $\pm$ 30	587 $\pm$ 30	0.240 $\pm$ 0.04	203.0 $\pm$ 18.0
HPD	30.0 $\pm$ 5.0 (-42%)	39.2 $\pm$ 4.6 (-20%)	1.68 $\pm$ 0.15 (+15%)	4.68 $\pm$ 2.47 (+12%)	117 $\pm$ 30 (-17%)	142 $\pm$ 58* (-76%)	0.146 $\pm$ 0.01* (-39%)	171.0 $\pm$ 16.0* (-16%)
<b>Liver</b>								
Control	42.8 $\pm$ 5.46	98.7 $\pm$ 3.88	7.62 $\pm$ 0.18	4.62 $\pm$ 0.18	60.70 $\pm$ 7.6	312 $\pm$ 12	ND	ND
HPD	27.2 $\pm$ 4.71* (-36%)	67.6 $\pm$ 3.51* (-32%)	10.05 $\pm$ 0.17* (+40%)	5.88 $\pm$ 0.14* (+27%)	86.44 $\pm$ 4.2* (+42%)	382 $\pm$ 8* (+22%)	ND	ND

Results are mean  $\pm$  SEM of five different preparations. Values in parenthesis represent percent change from control. \*Significantly different from corresponding control at  $p < 0.05$  or higher degree of significance by group  $t$  test and ANOVA. ND- not determined.

<sup>1</sup> LDH, lactate dehydrogenase; MDH, malate dehydrogenase; FBPase, fructose 1,6-bisphosphatase; G6Pase, glucose 6-phosphatase; G6PDH, glucose 6-phosphate dehydrogenase; ME, NADP-malic enzyme; ICDH, isocitrate dehydrogenase; HPD, high protein diet

## Discussion

Diet and nutrition play important roles in the maintenance of health. However, sugar, fat and protein-rich diets, fried and processed foods and so called popular or TV diets such as Atkins diet cause negative effects on human health (22-25). Primarily, they affect the structure and functions of intestine, liver and kidneys (5, 8, 9, 26). Although the effects of HCD and HFD on BBM enzymes and metabolic activity have been assessed in the intestine and liver, the effects of HPD have not been carried out in details, except for a few studies. To achieve further insights into the adaptive mechanisms of intestine and liver to dietary proteins, this study assessed the effects of HPD on various serum parameters and enzymes of BBM and energy-yielding metabolic pathways such as glycolysis, TCA cycle, gluconeogenesis HMP shunt in small intestine and liver of rats. These results were compared with those of a recently published report of the current authors on the effects of HCD and HCD (5). The present results demonstrated that HPD moderately increased the body weight similar to feeding of HCD. However, this was in contrast to HFD, which caused

decreasing in body weight (5). The HPD increased serum glucose and decreased inorganic phosphate; however, serum cholesterol and phospholipids were unchanged. The increase in serum glucose might be due to the conversion of proteins to glucose via gluconeogenesis.

Effects of HPD on the structural integrity of intestinal mucosa were assessed through the status of BBM marker enzymes of ALkPase, GGTase and sucrase in isolated BBM from mucosal homogenates. These hydrolytic enzymes are involved in the final stages of digestion and subsequent absorption. The activity of these enzymes was ten-fold higher in the BBM than in the respective homogenates, showing purity of the isolated BBM. The activity of ALkPase greatly increased (+101%) whereas GGTase activity moderately increased (+52%) in the BBM by HPD, verifying findings of previously published studies (8, 11-13, 27). In contrast, HPD significantly decreased (-37%) the activity of sucrase in the BBM as reported previously (6, 10). These results showed that the effects of HPD were adaptive alterations in the BBM enzyme activities based on the quantity of proteins and carbohydrates in the diets. Kinetic

studies revealed that the increases of ALkPase and GGTase activity were due to the increases in Vmax and decreases in Km values; however, decreases in sucrase activity were resulted from decreases in Vmax and Km values. It seemed that the diets might affect the number of enzyme molecules and change their characteristics.

The macromolecular complex food components are generally digested to small molecules such as glucose, AAs, fatty acids (FAs) and Pi, which are efficiently absorbed by specific transporters across intestinal BBM. The absorptive functions of small intestine involve vital energy-dependent transport processes, which are supported by various energy-yielding pathways of carbohydrate metabolism (e.g., glycolysis, TCA cycle, HMP shunt and gluconeogenesis). Although the effects of HCD and HFD on the metabolic activity of intestine and liver have been reported, long-term effects of HPD have not been investigated systematically in the intestine and liver. Raheja et al. (15) reported decreases in glucose oxidation by HPD in chick intestines. Another study reported that the increases of dietary protein and decreases of carbohydrates and lipids decreased the glycolytic activity and induced hepatic gluconeogenesis as a strategy to provide metabolic energy from AAs (16). The present results showed that HPD caused specific alterations in enzyme activities involved in various metabolic pathways. The HPD significantly decreased activities of LDH, MDH, SDH, ICDH, indicating a lower oxidation by glycolysis and TCA cycle in the intestine.

The activity of G6PDH and ME involved in the generation of NADPH decreased as well. The NADPH, which is needed for certain biosynthetic pathways and antioxidant defense mechanism, was affected. However, HPD increased gluconeogenesis as shown by increased activities of FBPase and G6Pase in the intestine. Moreover, the effects of HPD on liver metabolic activity were quite different, compared to that observed in the intestine. The activity of LDH and MDH increased and the activity of G6Pase and FBPase decreased by HPD in the liver. The activity of lipogenic enzymes of G6PDH and ME significantly increased by HPD in the liver. Thus, It is clear that the enzymes of glucose degradation and HMP shunt decreased; however, those of gluconeogenesis decreased by HPD in the intestine. In the liver, enzymes involved in glycolysis, TCA cycle and HMP shunt increased whereas those of gluconeogenesis significantly decreased. Significant decreases were seen in serum Pi levels by HPD, indicating lack of Pi available for metabolic activities and hence ATP formation as demonstrated by decreased activities of metabolic enzymes.

It is well established that antioxidant status can be used as a biomarker to assess chronic disease risks and diets can modulate antioxidant defense systems (7, 28). Although this study has not assessed the effects of HPD on oxidant-

antioxidant system, literature was searched and a few studies were detected regarding the effects of HPD on oxidant-antioxidant parameters. The authors have recently reported that HCD and HFD increased oxidative stress and decreased antioxidant parameters in the intestine and liver (5). However, long-term consumption of HPD does not increase variables of oxidative stress in the rat liver (29). In another study, Erdemli et al. (30) has reported that while HCD and HFD increased oxidative stresses in the kidneys, HPD included no effects on oxidative parameters.

In summary, the underlying mechanisms; by which, HPD caused significant alterations in serum parameters, BBM enzyme activities and overall metabolic health statuses seem complex. The HPD increased serum glucose and decreased inorganic phosphate; however, serum cholesterol and phospholipids were not affected. The activity of ALkPase and GGTase increased but that of sucrase decreased by HPD. Kinetic analysis revealed that the increase of ALkPase and GGTase activity was due to the increase in Vmax and decrease in Km values whereas the decrease in sucrase activity was due to the decrease in Vmax and Km values. In addition, HPD caused significant decreases in the activities of LDH, MDH, SDH, ICDH, indicating a lower oxidation rate of sugars by glycolysis and TCA cycle in the intestine. The activity of G6PDH and ME decreased. However, HPD increased gluconeogenesis as shown by increased activities of FBPase and G6Pase in the intestine.

The HPD decreased the activity of LDH and MDH whereas increased the activity of FBPase, G6Pase, G6PDH and ME in liver homogenates. It seemed that HPD lessened the glycolytic and TCA cycle activities and induced intestinal and hepatic gluconeogenesis as a strategy to provide metabolic energy from AAs available from digested proteins. Previous studies have suggested that HCD may not pose serious metabolic risks whereas HFD disrupts the homeostasis of cellular metabolism and is a key initiator of the metabolic disorder. Significant interests are reported for HPDs to manage weight control. Results of this study suggest that rats fed HPDs successfully adapt to the dietary protein concentration and maintained a healthy body weight. Therefore, HPD seems further effective for weight control management without metabolic risk factors, compared to that HCD and HFD do (5, 14).

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The authors declare no conflict of interest.

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### Abbreviations

ALkPase, alkaline phosphatase  
ATP, adenosine triphosphate  
BBM, brush border membrane  
BBMV, brush border membrane vesicles  
FBPase, fructose 1,6-bisphosphatase  
G6Pase, glucose 6-phosphatase  
G6PDH, glucose 6-phosphate dehydrogenase  
GGTase,  $\gamma$ -glutamyl transferase  
GSH-Px, glutathione peroxidase  
HCD, high carbohydrate diet  
HFD, high fat diet  
HPD, high protein diet  
HMP, hexose monophosphate pathway  
ICDH, isocitrate dehydrogenase  
LDH, lactate dehydrogenase  
LPO, lipid peroxidation  
MDA, malondialdehyde  
MDH, malate dehydrogenase  
ME, NADP-malic enzyme  
NAD, nicotinamide adenine dinucleotide  
NADH, nicotinamide adenine dinucleotide reduced  
NADPH, nicotinamide adenine dinucleotide phosphate reduced  
NADP, nicotinamide adenine dinucleotide phosphate  
ROS, reactive oxygen species  
SDH, succinate dehydrogenase  
SO, superoxide dismutase  
TCA, tricarboxylic acid

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