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Effect of Fasting on Enzymes of Carbohydrate Metabolism, Brush Border Membrane and on Transport Functions in Superficial and Juxta-Medullary Cortex of Rat Kidney

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Abstract

The effect of 1, 3 and 5-day fasting was studied on serum parameters; enzymes of brush border membrane and carbohydrate metabolism; transport of Pi and proline in different parts of the rat kidney. Fasting decreased the activities of lactate dehydrogenase, malate dehydrogenase but increased the activities of glucose-6-phosphatase and fructose 1,6-bisphosphatase; glucose-6-phosphate dehydrogenase and malic enzyme. These observations suggest that the degradation of glucose is decreased but its production by gluconeogenesis is enhanced upon fasting. Fasting led to significant decrease in the specific activities of Brush Border Membrane (BBM) enzymes, alkaline phosphatase and γ-glutamyl transferase in BBM vesicles prepared from superficial and juxta-medullary cortex. The transport of Pi was also decreased albeit differentially in these BBM preparations. Kinetic studies revealed that the activity of BBM enzymes and Pi transport decreased due to changes in V_{max} and K_m values. The results show that fasting caused significant decrease in metabolic enzymes involved in energy generation that led to decreased transport functions of the kidney.

Keywords: Kidney; Brush border membrane; Carbohydrate metabolism; Alkaline phosphatase

Abbreviations: AlkPase: Alkaline Phosphatase; BBM: Brush Border Membrane; BUN: Blood Urea Nitrogen; BBMV: Brush Border Membrane Vesicle; FBPase: Fructose 1,6-Bisphosphatase; G6PDH: Glucose: 6-Phosphate Dehydrogenase; GGTase: ɤ-Glutamyl Transferase; HK: Hexokinase; HMP: Hexose Monophosphate; JMC: Juxtamedullary Cortex; LDH: Lactate Dehydrogenase; MDH: Malate Dehydrogenase; ME: Malic Enzyme; NADPH: Nicotinamide Adenine Dinucleotide Phosphate Reduced; NADP: Nicotinamide Adenine Dinucleotide Phosphate; SC: Superficial Cortex; WC: Whole Cortex

Introduction

Nutritional stress, starvation, malnutrition and dietary restrictions due to socio-economic, life style or religious reasons can produce negative impact on human health. They have been shown to alter structure, metabolic activity and functional capabilities of kidney, liver and intestine [1-4].

The kidney is vital organ which plays essential role in the maintenance of body fluid volume, its composition and pH with in physiological range. The kidney is a heterogeneous structure consisting of discreet tissue zones e.g., cortex, the outer and inner medulla with individual "organ" characteristics with respect to structure, metabolism and functions [5-8]. The metabolic activity, the oxygen tension and the transport functions vary in different tissue of the kidney [9,10]. For example, the cortex is the site for oxidative metabolism and gluconeogenesis whereas anaerobic glycolysis is prevalent in the medulla [11]. Most solutes, ions and water are reabsorbed in the kidney by its proximal tubule across its BBM, which contains a number of hydrolytic enzymes and transport systems. These tubules function differentially in different cortical regions [5].

The kidney has the ability to adapt to the changes in the external and internal environment in acute situations for maximum work functions [12]. Fasting (starvation) is known to produce extensive changes in the structure and functions of intestine, liver and kidney [2,13]. Earlier we have reported that 1, 3 or 5 days fasting led to decrease in the activity of certain enzymes involved in terminal digestion and absorption of food components in rat intestine. The enzymes of glucose metabolism involved in glycolysis and HMP-shunt were also decreased but enzymes of gluconeogenesis increased by fasting [2]. However, Islamic fasting during the month of Ramadan increased many of those enzymes in the intestine and kidney and renal transport of Pi was also increased in experimental rats [3,4].

The aim of the present study was to determine the effect of 1, 3, and 5 day fasting on the enzymes carbohydrate metabolism and Brush Border Membrane (BBM) and on BBM transport of Pi and proline in different renal tissues i.e. in whole, superficial and juxta-medullary cortex to assess the structural integrity, metabolic activity and functional capability of the kidney. The results of the present study demonstrate that fasting caused decrease in the breakdown of glucose but enhanced its production by gluconeogenesis. The Pi transport capacity was also decreased. However, the kidney was able to maintain a positive balance of serum Pi by decreasing Pi excretion in the urine.

Material and Methods

Wistar rats were purchased from All India Institute of Medical Sciences (New Delhi, India). ^{32}Pi was purchased from Bhabha Atomic Research center (Mumbai, India). All other chemicals used were of analytical grade and were purchased from Sigma Chemical Co. (St Louis, MO, USA) unless otherwise stated.

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Experimental design

Adult male Wistar rats weighing 100-125 g, fed with a standard rat diet (Amrut Laboratories, Pune, India) and water ad libitum, were conditioned for one week before the start of the experiment. All animals were kept under conditions that prevented them from experiencing unnecessary pain according to the guide lines of Institution Ethical Committee. Rats were fasted for 1, 3 or 5 days. During fasting rats were deprived of food but were given ad libtum 1% glucose-water to reduce the mortality due to prolonged fasting (up to 5 days).The unfasted control group of rats received standard diet and 1% glucose water throughout the experiment. The experiment was designed in such a way that all of the animals (fasted and control) were sacrificed on the same day under light ether anesthesia. The weights of the animals were recorded at the starting and completion of the experiment. Blood and urine were collected and the kidneys harvested and utilized for further analyses.

Preparation of homogenates

After the completion of the experiment, the kidneys were removed, encapsulated and kept in ice-cold buffered saline (154 mM NaCl, 5 mM Tris-HEPES, pH 7.5). The whole cortex and superficial and juxta-medullary cortex were carefully separated as described earlier [5]. A 15% (w/v) homogenate was prepared in 0.1 M Tris-HCl buffer pH 7.5 using Potter-Elvehejem homogenizer (Remi motors, Mumbai, India) with five complete strokes. The homogenate was centrifuged at 3000 g at 4°C for 15 min to remove cell debris and the supernatant was saved in aliquots and stored at -20°C for assaying various enzymes.

Preparation of brush border membrane vesicles (BBMV) from various tissue zones of the kidney

BBMV were prepared from the homogenates of Whole Cortex (WC), Superficial Cortex (SC) and Juxta-Medullary Cortex (JMC) using the $MgCl_2$ precipitation method as previously described [6]. Briefly, freshly minced tissues were homogenized in 50 mM mannitol and 5 mM Tris-HEPES buffer pH 7.0 (20 ml/g), in a glass Teflon homogenizer with 4 complete strokes. The homogenate was then subjected to high speed homogenization in an Ultra Turex homogenizer (Type T-25, Janke & Kunkel GMBH & Co. KG. Staufen) for three strokes of 15 s each with an interval of 15 s. $MgCl_2$ was added to the homogenate to a final concentration of 10 mM and the mixture stirred for 20 min on ice. The homogenate was centrifuged at 2000 g for 10 min in a Beckman centrifuge (J2 MI, Beckman instruments Inc, Palo Alto, C.A. USA) using JA-17 rotor and the supernatant was then recentrifuged at 35,000 g for 30 min. The pellet was resuspended in 300 mM mannitol and 5mM Tris-HEPES, pH 7.4, with four passes by a loose fitting Dounce homogenizer (Wheaton IL, USA) and centrifuged at 35,000 g for 20 min in a 15 ml corex tube. The outer white fluffy pellet of BBMV was resuspended in small volume of buffered 300 mM mannitol. Aliquots of homogenates and BBMV were saved and stored at -20°C for BBM enzyme analyses. Each sample of BBMV was prepared by pooling tissues from 3-4 rats.

Serum chemistries

Serum samples were deproteinated with 3% trichloroacetic acid in a ratio 1: 3, left for 10 min and then centrifuged at 2000 × g for 10 min. The protein free supernatant was used to determine inorganic phosphate and creatinine. The precipitate was used to quantitate total phospholipids. Blood Urea Nitrogen (BUN) and cholesterol levels were determined directly in serum samples. Glucose was estimated by o-toluidene method using kit from Span diagnostics (Mumbai, India). These parameters were determined by standard procedures as mentioned in a previous study [4].

Enzyme assays

The activities of BBM biomarkers enzymes, alkaline phosphatase (AlkPase), γ-glutamyl transferase (GGTase) in the homogenates and BBM preparations were determined as described earlier [6]. The enzymes of carbohydrate metabolism, e.g., Lactate Dehydrogenase (LDH), malate dehydrogenase (MDH), glucose-6-phosphate (G6PDH) dehydrogenase and NADP-Malic Enzyme (ME), involved in oxidation of NADH or reduction of NADP were determined by measuring the extinction changes at 340 nm in a spectrophotometer (Cintra 5; GBC Scientific Equipment, Pty., Victoria Australia) as described elsewhere [4,7]. The other enzymes, glucose-6-phosphatase (G6Pase), fructose-1,6-bisphospatase (FBPase) and Hexokinase (HK) were determined as described in our previous studies [4]. Protein concentration was determined by the modified method of Lowry et al. [14].

Transport of ^{32}Pi

Measurement of ^{32}Pi (Bhabha Atomic Research Center, India) uptake in freshly prepared BBMVs was carried out at 25 °C by rapid filtration technique as described by Yusufi et al. [5] either in the presence or absence of Na-gradient. Uptake was initiated by addition of 30 µl of incubation medium containing 100 mmol/l mannitol, NaCl/KCl 100 mmol/l, 5 mmol/l Tris–HEPES, pH 7.5, 0.1 mmol/l $K_2H_{32}PO_4$ to 15 µl BBM suspension (50-100µg protein) and incubated for the desired time intervals (see Results). The uptake was stopped by the addition of 3 ml ice-cold stop solution (containing 135 mmol/l NaCl, 5 mmol/l Tris–HEPES and 10 mM sodium arsenate, pH 7.5) and filtered immediately through 0.45 µm DAWP Millipore (USA) filter and washed three times with the stop solution using a Cornwall-type syringe (Wheaton, IL). Correction for non-specific binding to filters was made by subtracting from all data the value of corresponding blank obtained by filtration of the incubation buffer without vesicles. The radioactivity of dried filters was measured by a liquid scintillation counter (Reckbeta, LKB, Wallac, Sweden) with 10 ml scintillation fluid (Cocktail T, SRL, India).

Statistical analyses

All data are expressed as Mean ± SEM for at least 4-5 different preparations. Statistical evaluation was conducted by one-way ANOVA and by unpaired student's t test using SPSS 7.5 software. A probability level of $p<0.05$ was selected as indicating statistical significance. All the changes were compared with control values for better understanding and clarity.

Results

In general, 1, 3, and 5 days fasting caused marked reduction of body weights (-37%). The kidney and cortex were also declined in parallel (data not shown).

Effect of fasting on serum and urine parameters

The results in Table 1 showed that serum and urine creatinine was not affected up to 5 days of fasting indicating normal functioning of kidney. However, serum inorganic phosphate (Pi) declined significantly in 5 days fasted rats while urinary Pi was progressively and significantly lowered by 3 and 5 days fasting (Table 1). Serum phospholipids (PLs) and Cholesterol (Ch) increased significantly upon 3 and 5 days fasting (Table 1).

Group/Parameters	(a) Serum				(b) Urine	
	Creatinine (µmoles/ml)	Phosphate (µg/ml)	Phospholipids (µg/ml)	Cholesterol (mg/ml)	Creatinine (µmoles/ml)	Phosphate (µg/ml)
Control	22.17 ± 2.42	2.61 ± 0.02	456.25 ±3.24	2.26 ± 0.05	34.07 ± 2.31	1.57 ± 0.05
1 day fasting	21.90 ± 1.15 (-1.22%)	2.44 ± 0.04 (-6.51%)	430.90 ± 37.50 (-5.56%)	2.44 ± 0.05 (+7.96%)	34.81 ± 6.32 (+2.17%)	1.52 ± 0.03 (-3.18%)
3 days fasting	21.00 ± 1.86 (-1.21%)	2.44 ± 0.09 (-6.51%)	577.50 ± 2.50* (+26.57%)	2.76 ± 0.08* (+22.12%)	32.01 ± 6.55 (-6.04%)	1.23 ± 0.08* (-21.6%)
5 days fasting	20.06 ± 0.93 (-9.52%)	2.20 ± 0.03* (-15.7%)	807.50 ±15.90* (+76.98%)	2.80 ± 0.05* (+23.89%)	32.15 ± 0.50 (-5.63%)	1.08 ± 0.02* (-31.2%)

Results are expressed as Mean ± SEM in each of the three different preparations
*Significantly different from control, p<0.05 by group t-test. Values in parentheses represent percent change from control values

Table 1: Effect of fasting on (a) serum and (b) urine parameters.

Effect of fasting on enzymes of carbohydrate metabolism in different regions of kidney homogenates

The effect of fasting was determined on the activities of enzymes belonging to various carbohydrate metabolic pathways viz. glycolysis, TCA cycle, gluconeogenesis and HMP shunt pathway in the homogenates of Superficial Cortex (SCH) and Juxta-Medullary Cortex (JMCH). The activity of LDH declined progressively in proportion to the duration of fasting in kidney. The effect of 1-5 days fasting on the activity of LDH was more pronounced in JMCH compared to SCH where the activity was lowered to 52.6% after 5 days fasting (Table 2b). MDH (TCA cycle) activity was similarly altered by fasting. The decreasing effect due to fasting was not significant after 1 day fasting both in SCH and JMCH. However, after 5 days fasting, the activity of MDH decreased significantly and the decline appeared to be slightly greater in JMCH (-48.4%) than in SCH (-41.4%). In contrast to glycolysis and TCA cycle enzymes, the activities of gluconeogenic enzymes (FBPase and G6Pase) were increased significantly by fasting (Table 2). The FBPase activity increased significantly in SCH (+51.4%) than insignificantly in JMCH (+6.2%) after 1 day fasting. The activities further increased after 3-5 days fasting (Table 2). In 5 days fasted rats, the activity of FBPase was profoundly increased in SCH (+92.3%) as compared to JMCH (+19.8%). Similarly, the increase in G6Pase activity due to fasting was much greater in SC (+93.7%) than in JMCH (+29.7%).

The activities of G6PDH and ME, that provide NADPH needed in various anabolic reactions, were also determined. The activity of G6PDH was increased significantly even after 1 day of fasting in SCH (+84.1%) and further increased to a much greater extent in SCH after 3 and 5 days fasting. The ME activity however, increased significantly only in JMCH (+32.7%) and not in SCH (+8.1%) in 1 day fasted rats. A maximum increase in ME activity was observed in JMCH (+61.9%) after 5 days fasting. Hence, the activity of both G6PDH and ME increased sharply in kidney homogenates by 1-5 days fasting.

Effect of fasting on the enzymes and transport properties of renal BBMV's isolated from different regions of kidney cortex

The effect of 1, 3 and 5 days fasting on the activities of BBM marker enzymes in the homogenates and BBM preparation and transport of ^{32}Pi and 3H-L-proline in BBMV isolated from Whole Cortex (WC), SC and JMC as described in the "methods".

Effect of fasting on BBM enzyme activities in the homogenate and BBMV

The activities of BBM marker enzymes viz. AlkPase and GGTase were determined in CH and BBMV-WC, BBMV-SC and BBMV-JMC (Table 3a). The specific activities of AlkPase and GGTase decreased both in homogenates and BBMV preparations. However, there was a greater decline in BBMV than in respective homogenates. Further analysis showed that AlkPase activity declined significantly in BBMV-SC (-28.9%) in 1 day fasted rats as compared to control rats. The activity was further decreased after 3 and 5 days fasting and the effect was linearly proportional to duration of fasting. However, after 5 days fasting, the decrease in activity was more prominent in BBMV-JMC (-44.2%) than BBMV-SC (-37.6%). In CH, the activity of AlkPase was significantly lowered in SCH (-26.8%) but not in WCH (-9.47%) and JMCH (-1.74%) after 1 day fasting. A significant decrease was observed in homogenates after 5 days fasting.

The activity of GGTase was differentially affected by fasting in the homogenates and BBMV (Table 3b). The effect of fasting on the activity of GGTase appeared to be dropped after 5 days as compared to 3 days fasting. However, the effect of 1-5 days fasting was more prominent in BBMV-JMC than in BBMV-SC. Moreover, in WCH and SCH, increased in the activity of GGTase is observed than the control values (Table 3b). The kinetic parameters (V_{max} and K_m) were also determined by assaying the enzymes in BBMV are isolated from different regions of kidney cortex at different substrate concentrations. As shown in Table 3, the effect of 1-5 days fasting on AlkPase and GGTase was due to alterations in both V_{max} and K_m (Table 4).

Effect of fasting on the transport of phosphate (^{32}Pi) and ^{3}H proline in BBMVs isolated from different regions of kidney cortex

The effect of 1, 3 and 5 days fasting was determined on transport of ^{32}Pi in BBMVs isolated from different regions of kidney cortex. As shown in Table 5, the transport of ^{32}Pi in the presence of Na-gradient (Na0>Nai) markedly lowered (-57.8% and -42.7%) by 1 day fasting in the initial uphill phase (5s and 20s). The transport further declined after 3 and 5 days fasting by -64.9% and -71.7% at 5s and -44% and -56% at 20s. The marked decrease in Na-dependent uptake of ^{32}Pi was also apparent when calculated as percent overshoot (Δ%). Similar to BBMV-WC, the Na- dependent ^{32}Pi transport in uphill phase (5 s and 20 s) was also lowered linearly with increasing duration of fasting (1-5 day) in BBMV-SC and BBMV-JMC. The effect of fasting on ^{32}Pi uptake is much greater in BBMV-SC than BBMV-JMC. The transport of ^{32}Pi, however, was not affected when determined at 120 min. either in presence (NaCl in the medium) or absence of Na-gradient (NaCl is replaced by KCl in the medium) in BBMV-WC, BBMV-SC and BBMV-JMC (data not shown). Kinetic analysis of ^{32}Pi transport indicates that the decrease in the uptake of ^{32}Pi by 1-5 days fasting was due to marked decrease both in V_{max} and K_m values (Table 6). The reduction in V_{max} values due to fasting was also greater in BBMC-SC compared to BBMV-JMC.

In the same preparations of BBMV's the Na-gradient dependent uptake of 3H-L-proline was also determined (Table 7). The uptake

Group/Enzymes	LDH	MDH	FBPase	G6Pase	G6PDH	ME
(a) SCH						
Control	44.70 ± 2.63	106.50 ± 0.39	9.38 ± 0.12	5.10 ± 0.20	214 ± 26.0	733 ± 23.0
1 day fasting	41.00 ± 0.35 (-8.2%)	101.20 ± 0.84 (-16.4%)	14.20 ± 0.48* (+51.4%)	6.32 ± 0.16* (+23.9%)	394 ± 4.0* (+84.1%)	806 ± 14.0 (+8.1%)
3 days fasting	36.01 ± 0.29*	82.90 ± 0.79*	16.70 ± 0.38*	6.53 ± 0.09*	476 ± 5.0*	815 ± 8.0*
	(-19.4%)	(-22.1%)	(+78%)	(+28%)	(+122.4%)	(+11.2%)
5 days fasting	26.70 ± 1.06*	62.70 ± 0.42*	18.04 ± 0.23*	9.88 ± 0.18*	614 ± 6.0*	936 ± 2.0*
	(-40.2%)	(-41.4%)	(+92.3%)	(+93.7%)	(+186.9%)	(+27.6%)
(b) JMCH						
Control	59.60 ± 1.56	130.90 ± 0.60	7.90 ± 0.17	4.00 ± 0.13	379 ± 6.0	821 ± 8.0
1 day fasting	49.80 ± 1.92	125.80 ± 0.28	8.39 ± 0.15	4.78 ± 0.09	441 ± 5.0*	1090 ± 1.0*
	(-16.4%)	(-3.89%)	(+6.2%)	(+19.5%)	(+16.3%)	(+32.7%)
3 days fasting	39.16 ± 1.38*	104.60 ± 1.15*	9.11 ± 0.21*	4.82 ± 0.04	484 ± 2.0*	1120 ± 14.0*
	(-34.2%)	(-20.1%)	(+15.3%)	(+20.5%)	(+27.7%)	(+36.4%)
5 days fasting	28.20 ± 1.27*	67.50 ± 1.00*	9.47 ± 0.06*	5.19 ± 0.12*	1390 ± 30.0*	1330 ± 14.0*
	(-52.6%)	(-48.4%)	(+19.8%)	(+29.7%)	(+266.7%)	(+61.9%)

Results (specific activity expressed as μmoles/mg protein/hr) are expressed as Mean ± SEM for three different preparations. Each preparations includes kidney of 3-4 animals in each group
*Significantly different from control, p<0.05 by group t-test. Values in parentheses represent percent change from control values

Table 2: Effect of fasting on the specific activities of lactate dehydrogenase (LDH), malate dehydrogenase (MDH), fructose 1,6-bisphosphatase (FBPase), glucose-6-phosphatase (G6Pase), glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme (ME) in a) superficial cortical homogenate (SCH) and b) juxtamedullary cortical homogenate (JMCH).

Group	WC		SC		JMC	
(a) AlkPase	**CH**	**BBMV**	**CH**	**BBMV**	**CH**	**BBMV**
Control	45.49 ± 2.07	276. 33 ± 4.15	19.72 ± 0.42	171.01 ± 4.26		147.02 ± 3.61
1 day fasting	41.18 ± 0.09	238.60 ± 1.39*	14.43 ± 0.13*	121.72 ± 2.07*	15.24 ± 2.72	114.53 ± 1.73*
	(-9.47%)	(-13.65%)	(-26.82%)	(-28.9%)	(-1.74%)	(-22.09%)
3 days fasting	40.69 ± 0.24	213.40 ± 0.64*	13.96 ± 0.23*	110.19 ± 2.53*	13.10 ± 1.90	101.21 ± 2.36*
	(-10.55%)	(-22.77%)	(-29.20)	(-35.5%)	(-15.53%)	(-31.15%)
5 days fasting	35.96 ± 0.13*	188.40 ± 0.68*	13.45 ± 0.18*	106. 68 ± 2.42*	10.71 ± 0.91*	82.01 ± 1.34*
	(-20.94%)	(-31.80%)	(-31.79%)	(-37.61%)	(-30.9%)	(-44.2%)
(b) GGTase						
Control	40.57 ± 1.59	328.46 ± 5.98	23.00 ± 0.03	189.95 ± 4.49	61.90 ± 0.48	547.90 ± 0.01
1 day fasting	40.00 ± 0.03	272.07 ± 1.94*	20.00 ± 0.02*	163.35 ± 0.81*	52.80 ± 1.40*	393.40 ± 0.60*
	(-1.40%)	(-17.17%)	(-13.04%)	(-14.0%)	(-14.70%)	(-28.19%)
3 days fasting	40.12 ± 0.01	200.75 ± 1.18*	23.40 ± 0.03	153.80 ± 4.38*	45.90 ± 2.19*	338.20 ± 1.38*
	(-1.10%)	(-38.88%)	(+1.74%)	(-19.03%)	(-25.85%)	(-38.27%)
5 days fasting	47.91 ± 0.51*	264.54 ± 1.80*	25.40 ± 0.04*	169.60 ± 4.53	58.00 ± 0.63	393.40 ± 1.20*
	(+18.09%)	(-19.46%)	(+10.04%)	(-10.71%)	(-6.30%)	(-28.19%)

Results (specific activity expressed as μmoles/mg protein/hr) are expressed as Mean ± SEM for three different preparations. Each preparations includes kidney of 3-4 animals in each group
*Significantly different from control, p<0.05 by group t-test. Values in parentheses represent percent change from control values

Table 3: Effect of fasting on the specific activities of (a) alkaline phosphatase (AlkPase) and (b) gamma glutamyl transpeptidase (GGTase) in cortical homogenates (CH) and brush border membrane vesicles (BBMV) from whole cortex (WC), superficial cortex (SC) and juxtamedullary cortex (JMC).

of 3H-L-proline was differentially affected by 1-5 days fasting. The initial uptake of L-proline at 20 s was significantly lowered both by 1 day (-22.6%) and 3 days (-40.3%) fasting bur increased after 5 days fasting in BBMV-SC and the values returned back toward the control values (Table 7). Na-gradient dependent uptake of L-proline was also determined in BBMV-SC and BBMV-JMC. The initial uphill uptake was determined at 20 s. The uptake of L-proline was markedly lowered by 1 and 3 days of fasting in both BBMV-SC and BBMV-JMC. However, after 5 days, the effect of fasting seems to be lowered and the values of uptake were higher in 5 days fasted than in 3 days fasted BBMV'S. Similar to ^{32}Pi, the effect of fasting on L-proline uptake was also found to be greater in BBMV-SC than in BBMV-JMC. The Na-dependent and Na-independent uptake of 3H-L-proline at equilibrium (120 min.) were not altered by 1-5 days fasting, indicating similar intravesicular volume in different groups of rats.

Discussion

The present research was carried out to gain comprehensive knowledge regarding adaptive adjustment in rat kidney in general and proximal tubules in particular to maintain a positive balance of various metabolites and Pi involved in energy production needed for various renal functions during prolonged fasting situation. 1-5 days fasting resulted in marked reduction of bodyweights. The kidney and cortex weights were also declined in parallel otherwise rats were active during fasting period. However, Serum and urine creatinine were not affected by 1-5 days fasting suggesting that the body adapts well and that normal kidney functions remained intact.

To evaluate the effect of fasting on energy metabolism, certain enzymes involved in glycolysis, TCA cycle, gluconeogenesis and HMP-shunt pathway were determined. Fasting caused significant decrease in

Group	BBMV-WC		BBMV-SC		BBMV-JMC	
(a) AlkPase	Vmax†	Km x 10^{-3} M	Vmax†	Km x 10^{-3} M	Vmax†	K_m x 10^{-3}
Control	125	0.285	111.1	0.555	90.94	0.526
1 day fasting	86.95	0.217	95.23	0.526	74.07	0.476
	(-30 %)	(-23.8 %)	(-14.3 %)	(-5.26 %)	(-18.5 %)	(-9.50 %)
3 days fasting	62.5	0.196	76.92	0.5	62.5	0.512
	(-50 %)	(-31.2 %)	(-30.7 %)	(-10 %)	(-31.2 %)	(-2.66 %)
5 days fasting	45.45	0.178	66.66	0.588	50	0.625
	(-63.6 %)	(-37.5 %)	(-40 %)	(+6 %)	(-45 %)	(+18.8 %)
(b) GGTase						
Control	444.4	2	200	1.11	571	1.66
1 day fasting	222.2	0.952	125	0.76	333.3	1
	(-50 %)	(-52.4 %)	(-37.5 %)	(-31.5 %)	(-41.6 %)	(-39.7 %)
3 days fasting	148.1	0.645	100	0.66	250	0.71
	(-66.6 %)	(-67.7 %)	(-50 %)	(-40.5 %)	(-56.2 %)	(-57.2 %)
5 days fasting	200	1.25	250	1.42	400	1.11
	(-54.9 %)	(-37.5 %)	(+25 %)	(+27.9 %)	(-29.9 %)	(-33.1 %)

K_m (Michaelis Menton constant), Vmax (maximal velocity of enzyme reaction)
† µmoles/mg protein/hr
Values in parentheses represent percent change from control values

Table 4: Effect of fasting on kinetic parameters of a) Alkaline phosphatase (AlkPase) and b) gamma glutamyl transpeptidase (GGTase) in whole cortex (WC), superficial cortex (SC) and juxtamedullary cortex (JMC).

Group	Na- gradient dependant (Na0> Nai)											
	BBMV-WC				BBMV-SC				BBMV-JMC			
	5 s	20 s	120 min	Δ %	5 s	20 s	120 min	Δ %	5 s	20 s	120 min	Δ %
Control	938.1 ± 38.9	1413.3 ±18.9	330.55 ± 12.7	327.5	537.30 ± 15.19	793.39 ±15.19	254.50 ±20.29	211.6	242.17 ± 15.3	609.73 ± 38.8	235.31 ± 3.78	149.5
1 day fasting	395.9 ±24.03*	810.29 ±17.2*	328.03 ±13.06	147	326.10 ± 27.40*	463.26 ±14.84*	225.10 ±0.70	105.8	195.31 ± 25.1*	413.28 ± 38.0*	230.40 ± 21.6	79.3
	(-57.8 %)	(-42.7 %)	-0.76%	-	(-39.3 %)	(-41.6 %)	(-11.5 %)	-	(-19.4 %)	(-32.2 %)	(-2.08 %)	-
3 days fasting	329.2 ± 2.04*	797.4 ± 69.6*	324.26 ±36.1	145.9	292.90 ± 14.60*	408.40 ±29.70*	234.40 ±8.40	74.2	105.41 ± 5.46*	398.43 ± 48.1*	205.60 ±19.7	93.7
	(-64.9 %)	(-43.7 %)	(-1.90 %)	-	(-45.5 %)	(-48.5 %)	(-7.89 %)	-	(-56.5 %)	(-34.7 %)	(-12.6 %)	-
5 days fasting	265.2 ± 37.1*	621.8 ± 13.7*	315.9 ±12.09	96.8	184.07 ± 30.70*	297.22 ±15.70*	218.41 ±20.10	50.5	97.91 ± 16.4*	230.87 ± 8.7*	200.48 ± 19.8	15.1
	(-71.7 %)	(-55.9)	(-4.43 %)	-	(-65.7 %)	(-62.5 %)	(-14.2 %)	-	(-59.6 %)	(-62.1 %)	(-14.8 %)	-

Results are expressed as Mean ± SEM for three different preparations
*Significantly different from control, p<0.05 by group t-test. Values in parentheses represent percent change from control values.
Δ % overshoot at 20 s determined as a percent change in uptake form 120 min.

Table 5: Effect of Na-gradient and K-gradient uptake of ^{32}Pi in brush border membrane vesicles (BBMV) from whole cortex (WC), superficial cortex (SC) and juxtamedullary cortex (JMC).

Group	BBMV-SC		BBMV-JMC	
	V_{max}†	K_m x 10^{-3} M	V_{max}†	K_m x 10^{-3} M
Control	2352.9	1.37	1538.4	0.95
1 day fasting	1176.4	0.76	888.8	0.54
	(-50 %)	(-44.5 %)	(-42.2 %)	(-43.2 %)
3 days fasting	727.2	0.52	533.3	0.36
	(-69.1 %)	(-62 %)	(-65.3 %)	(-62.1 %)
5 days fasting	563.3	0.4	470.5	0.32
	(-76 %)	(-70.8 %)	(-69.4 %)	(-66.3 %)

K_m : Michaelis Menton constant; Vmax : maximal velocity of enzyme reaction
† pmoles/mg protein/10 s
Values in parentheses represent percent change from control values

Table 6: Effect of fasting on kinetic parameters of Na-dependant ^{32}Pi uptake as a function of an external Pi concentration by brush border membrane vesicles (BBMV) from superficial cortex (SC) and juxtamedullary cortex (JMC).

the activity of LDH and MDH in Superficial (SC) and Juxta-Medullary Cortex (JMC) similar to the effect observed in rat intestine [2]. The decrease was linearly proportional to fasting time. The effect on LDH seems significantly higher in JMC compared to SC as anaerobic glycolysis is more prevalent in the medulla than cortex. In contrast, activity of gluconeogenesis enzymes, FBPase and G6Pase profoundly increased by 1-5 days fasting also similar to the effect observed in intestine [2]. The enzymes of glucose metabolism and glucose synthesis are differentially distributed in the cortex and medulla. The oxidative metabolism and gluconeogenesis is more prevalent in superficial cortex whereas anaerobic glycolysis in juxta-medullary region [1,11]. The reduction in the activities of LDH and MDH suggests that the breakdown of glucose by both anaerobic and aerobic processes was slowed down in the absence of food intake. However, the synthesis

Group	Na-gradient dependant (Na0 >Nai)								K-gradient dependant (K_0>K_i)
	BBMV-WC		BBMV-SC			BBMV-JMC			BBMV-WC
	20 s	120 min	20 s	120 min	Δ %	20 s	120 min	Δ %	120 min
Control	15.20 ± 0.21	14.25 ± 1.40	86.56 ± 6.58	31.21 ± 4.88	177	57.7 ± 5.41	30.51 ± 9.50	149.5	14.70 ± 1.81
1 day fasting	11.76 ± 0.49*	13.12 ± 0.39	58.93 ± 6.58*	32.49 ± 3.59	81.3	45.71 ± 2.20*	31.17 ± 4.02	46.6	13.30 ± 1.69
	(-22.6 %)	(-7.92 %)	(-31.9 %)	(+4.10 %)	-	(-20.7 %)	(+2.16 %)	-	(-13.3 %)
3 days fasting	9.07 ± 0.51*	11.84 ± 0.55	43.21 ± 6.54*	30.34 ± 6.54	42.4	33.77 ± 3.69*	26.6 ± 2.27	26.9	12.25 ± 0.53
	(-40.3 %)	(-16.9 %)	(-50 %)	(-2.78 %)	-	(-41.4 %)	(-12.8 %)	-	(-16.7 %)
5 days fasting	14.53 ± 0.85	13.53 ± 1.24	62.41 ± 8.14*	27.95 ± 8.14	123.2	52.5 ± 6.41	25.47 ± 1.74	101.6	17.10 ± 0.78
	(-4.40 %)	(-5.05 %)	(-27.89 %)	(-10.44 %)	-	(-9.0 %)	(-16.5 %)	-	(+16.3 %)

Results are expressed as Mean ± SEM for three different preparations
*Significantly different from control, p<0.05 by group t-test. Values in parentheses represent percent change from control values.
Δ % overshoot at 20 s determined as a percent change in uptake form 120 min.

Table 7: Effect of fasting on Na-gradient dependant and K-gradient dependant uptake of ^{3}H-L-Proline in brush border membrane vesicles (BBMV) from whole cortex (WC), superficial cortex (SC) and juxtamedullary cortex (JMC).

of glucose by gluconeogenesis was increased most likely from the metabolites of fat and/or protein degradation as an adaptive mechanism under the prevailing situations arises by fasting.

Fasting also markedly increased the activities of G-6-PDH and NADP-malic enzymes. The increased production NADPH by these enzymes may have increased lipid biosynthesis and might be responsible for higher serum cholesterol and phospholipids. Since cholesterol and phospholipids are essential membrane components they may have been involved in repair and regeneration of cellular membranes affected by prolonged fasting. Increased cellular NADPH could also be utilized to reduce oxidative stress as has been observed in intestine and liver by fasting [15].

In addition to enzymes of glucose degradation, the activity of BBM marker enzymes; AlkPase and GGTase was also decreased although differentially in the homogenates and BBMV prepared from different kidney tissues. The effect was proportional to duration of fasting and maximum effect was observed after 5 days of fasting. Kinetic analysis conducted on BBMV prepared from WC, SC and JMC revealed that the decrease in enzyme activities in BBMV-SC was due to decrease in both V_{max} and K_m values but it was differentially observed In BBMV-SC and BBMV-JMC. The decrease in AlkPase was mainly due to V-max effect whereas that for GGTase was due to both V_{max} and K_m effect. Further, the alterations in kinetic parameters were different in BBMV-SC and BBMV-JMC. It has been established that AlkPase and GGTase are differentially located and organized in BBM; while AlkPase is considered to be marker for BBMC-SC, GGTase to be that of BBMV-JCM [5]. Thus these enzymes appeared to be differentially affected by fasting due to their location and organization in different tissues. The effect of fasting was also different on intestine and kidney BBM enzymes [2,3].

Pi is required for the production of metabolites and ATP in fact is conserved by the kidney. The bulk of Pi is transported in a Na-gradient-dependent manner by renal proximal tubule across its BBM and is an essential and regulatory step [16,17]. It is regulated by dietary Pi status, hormones and drugs [4,5,18,19]. The Pi transport was also shown to diffentially by total fasting and dietary Pi deprivation [20]. It has been shown that Na-gradient dependent Pi transport differs in BBMV-SC and BBMV-JMC [5,21]. It appears from the data that Pi is slightly decreased by 5 days fasting along with decrease in glucose metabolism that may lead to lower ATP production and hence Pi transport by the kidney. In confirmation of this notion, the present results show that Na-gradient dependent transport of Pi in uphill phase at 5s and 20s significantly decreased by 1-5 days fasting in both BBMV-SC and BBMV-JMC albeit differentially in proportional to the duration of fasting. The effect of fasting on Pi uptake was not observed at 120 min (equilibrium phase) and in the absence Na-gradient. Kinetic analysis revealed that decrease in the Pi transport was due to decrease of both V_{max} and K_m values. This decrease in Pi transport can be attributed to the decrease in the number of Na-gradient-dependent Pi transporters and in part due to alterations in the intrinsic properties of the transporters as observed in many pathological conditions [8,18]. Recently, oxidative stress and reduction in several growth factors are considered to be involved in fasting-induced deleterious effects [15,22,23].

In summary, the result of present study show that fasting caused extensive but specific adaptive changes in the metabolic activities and transport functions in the rat kidney. Fasting caused decrease in glucose break but increased its production by gluconeogenesis. Na-gradient dependent transport of Pi and L-proline were also decreased by fasting. However, the kidney tries to conserve Pi as indicated by only a small decrease in serum Pi and decrease of Pi excretion in the urine. We conclude that differential metabolic changes and transport functions in various kidney tissues can be attributed to the inactivation, decreased synthesis and/or increased degradation of various components under acute fasting conditions.

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