DISCUSSION
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It is well known that the kidney does not work to its full capacity under normal physiologic condition and it has the ability to adapt for its best capacity but differentially under many acute situations. The effect of dietary phosphorus intake on the structure and the functions of the kidney and especially on body Pi homeostasis is well characterized (5,31-39). The effect of starvation on the cellular metabolism and transport functions have been demonstrated (1,2,6,51,152-156). The dietary intake of high protein and high carbohydrate were shown to alter metabolic activity of the kidney (157-159). Effects of various drugs and hormones were also observed on the structure and certain functions of the kidney (32,35,39,93,98-122). Since the kidney is a heterogenous structure consisting of several tissue zones which inhabit internally in different environmental situations e.g. O$_2$ tension (PO$_2$) and composition of metabolites, therefore exhibit diverse metabolic activity and functional capacity. Additionally, the kidney is comprising of structurally and functionally distinct subsegments of the nephron, the fundamental unit of the kidney. The transport functions of the kidney are
largely dependent on the availability of ATP, the source of energy provided by various metabolic pathways. There appears to be a direct or indirect link between the metabolic activity and the function of especially the proximal tubular part of the kidney. Proximal tubule is the major regulatory metabolic and functional site in the kidney (26-30,40-42).

In view of the above, the present work was undertaken to study in greater detail the effects of hunger (starvation), religious compulsion (Islamic fasting) and dietary imbalances (high carbohydrate, high protein and high fat) on the

(a) General body conditions,
(b) serum and urinary biochemical parameters,
(c) activities of certain enzymes of carbohydrate metabolism in whole (WC), superficial (SC) and juxtamedullary cortex (JMC).
(d) activities of marker BBM enzymes (AlkPase and GGtase) in the homogenates and BBMV(s) isolated from WC, SC and JMC.
(e) transports of $^{32}$Pi and/or $^{3}$H-L-proline in BBMV-WC, BBMV-SC and BBMV-JMC.
1. **General**

The results indicate that 1, 3 and 5 d fasting resulted in the loss of body weights (Table 1) and they were partially regained when 2 d fasted rats were refed for 2 d. The kidney and cortex weights were also lowered by fasting and regained partially by refeeding. On the other hand, the loss of body and kidney weights by IF and HFD (Table 19, 43) was relatively small. However, the body and the kidney tissue weights were significantly increased by the feeding of HCD and HPD (Table 31).

2. **Serum and Urinary biochemical parameters**

Serum and urinary contents of creatinine were not altered by fasting, refeeding, IF, HCD, HPD or HFD, an indicator of normal kidney functions under these conditions. However, serum and urinary Pi were significantly but differentially altered by various dietary manipulations (Tables, 2, 12, 20, 32, 44). Both serum and urine Pi were significantly decreased by 3 and 5 d fasting but not by 1 d fasting (Table 2A & B). However, their levels were slightly (but not completely) restored upon refeeding (Table 12A & B) as also reported earlier (119). In contrast, IF, HCD and HFD caused significant increases in serum Pi levels while
urinary Pi levels were decreased under these conditions (Table 20, 32 & 44). On the other hand, serum Pi was lowered while urinary Pi increased by HPD (Table 32).

The effect on serum PL and Ch in many of above situations was not characterized earlier. The present results indicate that both PL and Ch were initially decreased in the serum by 1 d fasting but increased significantly by 3 and 5 d fasting (Table 2A). This increase in serum PL and Ch appeared to be due to the release in the blood by degeneration of membrane components and/or of lipoprotein hydrolysis. In contrast, the serum PL was lowered by IF without any change in the Ch content (Table 20A). On the other hand, both PL and Ch were increased by HFD (Table 44A) as also reported earlier (187) but not affected by HCD or HPD (Table 32A). Thus it appears that serum parameters may alter by various dietary stresses differentially according to the prevailing metabolic situations and their need.

3. Enzymes of carbohydrate metabolism

Since the kidney is a heterogenous structure consisting of distinct cortical and outer and inner medullary tissue zones, the enzymes of various pathways namely glycolysis,
TCA cycle, HMP-shunt pathway and gluconeogenesis have been found to be differentially distributed in these different tissue zones (1,12). It has been well established that activity of above pathways directly or indirectly link with the overall functioning of the kidney (188). Renal function especially the tubular functions are characterized as energy consumic transport processes which are dependent on the availability of energy provided by such metabolic pathways one way or the other (188).

In general, the metabolic pathways for carbohydrate metabolism have been described to be affected by the presence of oxygen-tension ($P_2O_2$) in different kidney tissue zones besides other factors, namely drugs, hormones and physiological and development state (17-20,23,46-49,64,67,73-78,119).

The present results indicate that the activity of both LDH and MDH (enzymes of glycolysis and TCA cycle respectively) significantly lowered by fasting, IF, HPD and HFD while significantly increased by HCD in the kidney and also in the liver (Table 5, 14, 23, 35, 47). The decrease in LDH by IF was comparatively less prominent (Table 23).
Further analyses showed that the decrease in the activity of both the enzymes by fasting was similarly higher in SC-H than JMC-H, while these enzymes were differentially altered by IF, HPD, HFD and HCD. In contrast to fasting, the activity of LDH was profoundly lowered in JMC-H by IF, HPD and HFD and largely increased in JMC-H by HPD. However, the activity of MDH, in contrast to LDH, lowered to much greater extent in SC-H by IF, HPD and HFD and increased also in SC-H by HCD (Table 23B, 35B & 47B). The activity in JMC-H was either not affected significantly or changed very little (Table 23B, 35B & 47B). It has been demonstrated that glycosis is predominant in deep cortex or medulla while TCA cycle (oxidative metabolism) is more prevalent in the cortex more so in the superficial cortex (2,50,51,52). The above enzymes were also altered in the liver but to different extent than the kidney and differentially by different dietary conditions. While fasting, IF and HFD produced lesser effect in the liver, greater effects were observed by HCD and HPD. The lowering effects by fasting, IF, HPD and HFD on LDH and MDH activity may reduce the generation of ATP thus altering the transport processes in the different kidney tissue zones accordingly.
It has been shown that carbohydrates are utilized first during fasting and other such conditions and then the supply of energy is maintained by the metabolism of fats and proteins. It has been also demonstrated that under such conditions glucose is synthesized from non-carbohydrate sources by gluconeogenesis (1,2,51,154,155).

As a result of the above fact, the activities of FBPase and G6Pase (enzyme of gluconeogenesis) were found to be increased by fasting. The increase of both the enzymes was found to be much higher in SC-H than JMC-H (Table 6A & B). This is in agreement with some of the previous reports (1,2,57,152-156). It is also known that gluconeogenesis is predominant in the proximal convoluted tubule (as could be present in SC-H) than in the proximal straight tubule is pars recta (as could be in JMC-H) (51,76).

The activities of both FBPase and G6Pase were differentially affected by IF, HCD, HPD and HFD as the metabolic conditions expected to be different under these situations. The activity of FBPase (+124%) was profoundly increased by IF but G6Pase activity was only moderately increased (+21%). The activities of the enzymes were also increased by HPD and HFD but not to the same extent as
observed by fasting (Table 6, 36, 48). However, both the enzymes were increased to similar extent. In contrast the enzymes were decreased by HCD (Table 36). The increase in the activity by fasting, IF, HPD or HFD or decrease by HCD was much greater in SC-H than JMC-H similar to the effect observed on MDH activity. IF had no significant effect on gluconeogenic enzymes of the liver while differential than the effect on kidney enzymes was observed on the liver by HCD, HPD and HFD. Previous studies also reported increased glycolysis and decreased gluconeogenesis in the proximal tubule by HCD (157), while increased gluconeogenesis and decreased glycolysis was characterized by HPD (158,159).

Glucose is also known to be oxidized though at a low rate by HMP-shunt pathway. However, the enzymes of this pathway together with malic enzyme (ME) have an important role in the production of NADPH which is being utilized by the kidney in drug metabolism, glutathione handling and in the synthesis of lipids and other membrane components (74,75,76). The activity of G6PDH was profoundly increased in the kidney and the liver by fasting as well as by IF (Table 7, 16, 25). The activity of this enzyme was also significantly increased in the kidney by HPD while decreased
by HCD and HFD. In the liver, the activity of G6PDH was increased by both HCD and HPD but decreased by HFD (Tables 37 & 49). The enzyme was profoundly altered in SC-H as compared to JMC-H by fasting, IF and by the diets. The activity of ME, however, was differentially affected by the conditions used in the study (Table 7B, 16B, 25B, 37B, 49B). The enzyme activity was increased significantly but to lesser extent than G6PDH by fasting and HPD while decreased in the kidney by IF, HCD and HFD (Table 7, 16, 25, 37, 49). The effect on ME activity under all the conditions appeared to be much greater in JMC-H than in SC-H (Table 7, 16, 25, 37, 49). On the other hand the activity of ME in the liver was significantly increased by fasting, IF, HCD and HPD while significantly decreased by HFD (Table 7B, 16B, 25B, 37B, 49B).

The results of the present study thus clearly demonstrate that the metabolic activity in the kidney and also in the liver is affected differentially in response to different nutritional conditions. The effect was also differentially observed in the different kidney tissue zones.
4. **Marker BBM-enzymes**

To observe the effect of various nutritional conditions on the structural components of the proximal tubules, the activity of marker BBM enzymes, namely AlkPase and GGTase in the homogenates and in the isolated BBMV(s) from WC, SC and JMC was determined. These enzymes are considered not only to structural importance but also to the functional one as they are implicated directly or indirectly in the transport of Pi and amino acids respectively (116-118). Further, these enzymes are differentially distributed in BBMV-SC and BBMV-JMC (156-189). Moreover, the activity of AlkPase was found to be increased under dietary Pi deprivation (LPD) and decreased by high phosphate diet (38,120,141,162). Also the activity of AlkPase was found to be changed in parallel in the same directions as the changes occurred in the transport of Pi in many albeit not in all the situations (116-118). However, direct involvement of this enzyme in the transport of Pi was evidently ruled out (41,138). There appeared to be a link between serum Pi and AlkPase activity as in many (but not in all) cases, the activity of AlkPase and serum Pi change in parallel (38,119,141,190,191). However, Pi deprivation by LPD causes an opposite affect in which serum Pi and AlkPase activity both increase (162,192,193).
The results of the present study indicate that AlkPase activity declined by 1, 3 and 5d fasting and the decrease was in proportion to the duration of fasting. The maximum decline was observed after 5 d fasting (Table 3A). The activity of GGTase was also declined by 1 and 3 d fasting. However, it was significantly increased by 5 d fasting. Kinetic studies revealed that both Vmax and Km were altered accordingly to much greater extent. The activity of AlkPase was maximally altered in BBMV-SC while GGTase was changed in BBMV-JMC by fasting (Table 3A & B).

The effect of IF was differentially observed on the activities of AlkPase and GGTase in BBMV-WC, BBMV-SC and BBMV-JMC (Table 21A & B). The activities of AlkPase and GGTase were lowered to similar extent in BBMV-WC by IF. However, the activity of AlkPase was profoundly decreased in BBMV-SC than BBMV-JMC while GGTase was lowered to much greater extent in BBMV-JMC than BBMV-SC (Table 21A & B). The activity of AlkPase was also lowered in BBMV-WC by HPD and HFD but increased by HCD (Table 33 & 45). In contrast, the activity of GGTase similar to fasting and IF declined by HCD and HFD but increased by HPD. The increase or decrease in the activity of AlkPase was always most profound in BBMV-SC.
while the changes in GGTase activity were always more pronounced in BBMV-JMC under various nutritional conditions. While both Vmax and Km were altered by fasting and IF, the effects by HCD, HPD and HFD were largely due to the alteration of Vmax values (Table 22, 34, 46).

5. Transport of $^{32}$Pi and/or $^3$H-L-proline

The reabsorption of Na by active transport appeared to be a major work function of the kidney as the transports of various solutes, ions and minerals are dependent on Na-reabsorption in the proximal tubules (40-45). The transport of $^{32}$Pi which occur in PT at BBM step is also regulated at this step by various conditions including by drugs, hormones and dietary intake of Pi (32,98-111).

Several intrinsic as well as extrinsic factors such as affinity (Km) and turnover rate (Vmax) of the transport, the concentration gradients of both solutes and the driving ions and the modulators (inhibitors or stimulators) are known to affect the renal handling of Pi (32,98-111). Further the effects were differently observed in BBMV(s) isolated from superficial (SC) and juxtamedullary cortex (JMC) due to the heterogeneity in structure as well as in functions of the PT-subpopulations (194).
Many hormonal and non-hormonal factors controlling Pi homeostasis have been shown to modulate the rate of BBM Na/Pi cotransport (136, 170, 194, 195). In general most in vivo maneuvers which modulate Pi reabsorption, so that the effect is retained at the BBM level. Some of the effects were shown to be dependent on the protein synthesis while others were not (194, 196). Thyroid hormones (T3) and dietary Pi deprivation by LPD stimulate BBM Na/Pi cotransport by Vmax effect but by different mechanism (197). The effect of both T3 and LPD was also different in BBMV-SC and BBMV-JMC (100, 105), PTH, calcitonin, high phosphate diet, nicotinamide, ANF, and acidosis were shown to inhibit Na/Pi cotransport also by different mechanism (32, 98–102, 113, 115).

As reported earlier by Kempson et al (119), Na-gradient dependent initial \(^{32}\)Pi transport in BBMV-WC was lowered by 1, 3 and 5 d fasting (Table-8). The maximum effect was observed by 5 d fasting, however \(^{32}\)Pi uptake was markedly lowered by 1 d fasting. The effect of fasting was reversed by refeeding (Table 17). Both Vmax and Km were greatly altered. The effect of fasting was much greater in BBMV-SC than BBMV-JMC (Table 9&18). However, in the same preparation
of BBMV(s) the uptake of L-proline was differentially affected by 1, 3, and 5 d fasting. The initial uptake of L-proline was significantly lowered by 1 and 3 d fasting but significantly increased by 5 d fasting as compared to 3 d fasted rats (Table 10). Interestingly, the activity of GGTase was also similarly affected by 1, 3 and 5 d fasting. This may suggest that at least in the case of fasting the activity of GGTase and the uptake of L-proline changed in parallel and in the same directions (Table 3A). GGTase was proposed to be directly or indirectly linked with the transport of amino acids (198,199). The results are also supported by the kinetic analysis (Table 4B).

In contrast to fasting, the of uptake $^{32}$Pi was significantly increased in BBMV-WC by 30 d Islamic fasting. The increase in $^{32}$Pi uptake was accompanied by a simultaneous increase in serum Pi while urinary Pi was decreased significantly (Table 26,20A&B). This observation is in contrast to the effect of fasting. However, kinetic parameters indicate that both Vmax and Km values were profoundly increased by IF. Unlike fasting, the increase in $^{32}$Pi uptake by IF was profoundly observed in BBMV-SC and the uptake in BBMV-JMC was only slightly increased (Table 28).
Kinetic studies showed similar observation in which both Vmax and Km values were predominantly increased in BBMV-SC and slightly in BBMV-JMC (Table 29).

Similar to the effect of Islamic fasting, Na-gradient dependent $^{32}$Pi uptake in the initial uphill phase was also markedly increased by HCD and moderately enhanced by HFD in BBMV-WC while significantly decreased by HPD (Table 38,50). Both Vmax and Km were markedly increased by HCD and HFD but decreased by HPD in BBMV-WC. The results further indicate that the alterations of $^{32}$Pi uptake were most pronounced in BBMV-SC and only small alterations were observed in BBMV-JMC by IF, HCD, HPD and HFD (Table 26,28,38,40,50,52). Under all these conditions both Vmax and Km were greatly altered however, the changes observed in the Vmax were greater than in the Km values. Further the changes observed in the uptake of $^{32}$Pi were in the same direction with the changes in serum Pi levels.

In several ways, the effects of IF, HCD and HFD on $^{32}$Pi uptake appeared to be similar to the observed effect of dietary Pi-deprivation while the effects of fasting and HPD appeared to be similar to the effect of high phosphorus diet (31,119,120,162). This also appears from the results of
present study as whole, that some nutritional conditions appear to be detrimental namely, fasting and to some extent HPD while others such as IF, HCD and to some extent HFD appear to be beneficial for the metabolism and transport of $^{32}\text{Pi}$ in the kidney in particular and for health in general.