Adenosine Triphosphate Turnover in Humans

Decreased Degradation during Relative Hyperphosphatemia

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Abstract

The regulation of ATP metabolism by inorganic phosphate (Pi) was examined in five normal volunteers through measurements of ATP degradation during relative Pi depletion and repletion states. Relative Pi depletion was achieved through dietary restriction and phosphate binders, whereas a Pi-repleted state was produced by oral Pi supplementation. ATP was radioactively labeled by the infusion of [8-^{14}C]adenine. Fructose infusion was used to produce rapid ATP degradation during Pi depletion and repletion states. Baseline measurements indicated a significant decrease of Pi levels during phosphate depletion and no change in serum or urinary purines. Serum values of Pi declined 20 to 26% within 15 min after fructose infusion in all states. Urine measurements of ATP degradation products showed an eightfold increase within 15 min after fructose infusion in both Pi-depleted and -supplemented states. Urinary radioactive ATP degradation products were fourfold higher and urinary purine specific activity was more than threefold higher during Pi depletion as compared with Pi repletion.

Our data indicate that there is decreased ATP degradation to purine end products during a relative phosphate repletion state as compared to a relative phosphate depletion state. These data show that ATP metabolism can be altered through manipulation of the relative Pi state in humans.

Introduction

ATP is intricately interwoven into cell energy transfer and intermediary metabolism (1–4). Therefore, perturbations in ATP metabolism may modify cellular function and lead to specific disease states. Since ATP synthesis requires inorganic phosphate in addition to oxygen, ADP, and NADH, phosphate concentrations in vivo may be rate limiting for ATP synthesis. Consequently, a phosphate-deprived state may be associated with decreased ATP synthesis and increased formation of ATP degradation products. Indeed, hypophosphatemia increases ATP degradation products in skeletal muscle (5, 6) and is correlated with a myriad of clinical manifestations including hemolytic anemia, respiratory failure, myopathy, and neurologic abnormalities in humans (7–14).

To elucidate the role of phosphorus, the regulation of ATP metabolism was examined during relative phosphorus depletion and repletion in normal volunteer subjects. ATP degradation was acutely activated by a previously developed model system using a rapid intravenous infusion of fructose (1–4, 15). A transient decrease of intracellular inorganic phosphate accompanies this transient intracellular ATP degradation. Under the conditions of this experiment a relative phosphorus-depleted state was associated with increased formation of ATP degradation products as compared to a relative phosphorus-repleted state.

Methods

Alucaps were obtained from Riker Laboratories, St. Paul, MN. Neutraphos was obtained from Willen Drug Co., Baltimore, MD. A 20% fructose solution was prepared by Central Pharmacy, University of Michigan Hospitals. Cathivex filter units (0.22 μM) were obtained from Millipore Corp., Bedford, MA. [8-^{14}C]Adenine and ACS scintillation fluid were obtained from Amersham Corp., Arlington Heights, IL.

Study design. ATP turnover was studied in vivo by using [8-^{14}C]adenine to label ATP (16). Radioactively labeled inosine, hypoxanthine, xanthine, and uric acid originate from ATP degradation and are excreted into the urine (Fig. 1). Under these conditions, increased quantities of unlabeled purines must come from another pathway, de novo purine synthesis, and can be distinguished from labeled compounds originating from ATP degradation. Rapid infusion of fructose causes the acute degradation of hepatic and renal ATP by its consumption in the phosphorylation of fructose (1–4, 15). The infusion of fructose was performed under states of relative phosphate depletion and repletion to study the effect of phosphate levels upon ATP degradation.

ATP synthesis occurs in the mitochondria by the following reaction: $3\ ADP + NADH + H^+ + 3Pi + 4O_2 \rightarrow 3\ ATP + 4H_2O + NAD^+$. If phosphate (Pi)[1] is rate-limiting for ATP synthesis, then relative phosphate depletion will decrease the rate of its resynthesis from ADP. Under these conditions more ADP will degrade to purine breakdown products rather than be converted back to ATP when the system is stressed by massive ATP utilization. Increased radioactively labeled purines originating from ATP should appear in the urine during relative phosphate depletion.

Five healthy male volunteers, ages 22 to 25 participated in this study. The volunteers selected had no history of medical problems, were taking no medications regularly, and had normal serum electrolytes, liver function tests, and hematocrit values. Informed consent was obtained according to the guidelines of the Human Subjects Review Committee of the University of Michigan.

ATP degradation was produced by intravenous fructose under three distinct states of phosphate intake. (*State a*) A relative phosphate-depleted state achieved through phosphate binders and a low-

Portions of this work were published in abstract form. (1988. *Arthritis Rheum.* 31:S109.).

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Received for publication 3 December 1988 and in revised form 4 May 1989.

J. Clin. Invest.

0021-9738/89/09/0990/06 $2.00
Volume 84, September 1989, 990–995

1. *Abbreviations used in this paper:* Pi, inorganic phosphate.

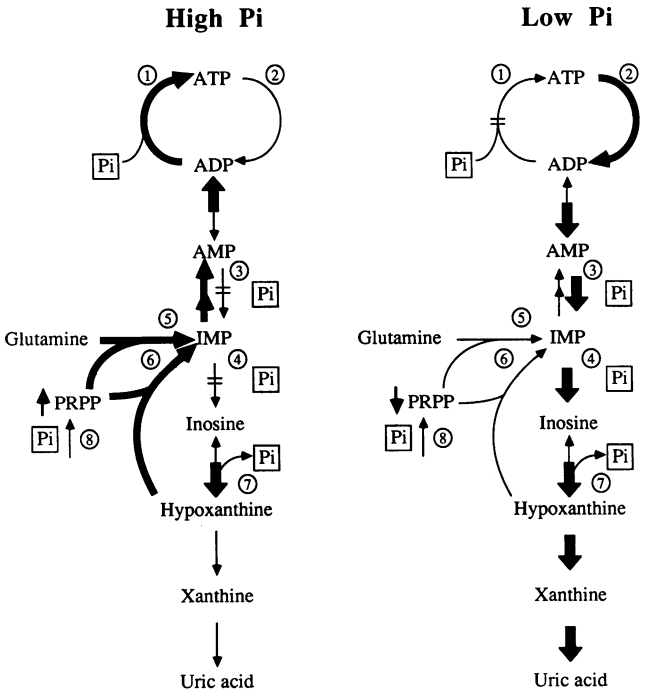

Figure 1. Inorganic phosphate (Pi) related regulation of ATP metabolism. This diagram contrasts the properties of high Pi compared to low Pi on ATP metabolism. Under high Pi conditions there is a net stimulation of ATP synthesis. High Pi levels stimulate purine nucleoside phosphorylase to convert inosine to hypoxanthine (reaction 7). They activate PRPP synthetase (reaction 8) which leads to increased formation of PRPP. Increased PRPP levels activate IMP synthesis through a stimulation of de novo purine synthesis (reaction 5) and hypoxanthine-guanine phosphoribosyltransferase (reaction 6). There is relative inhibition of AMP deaminase (reaction 3) and high K_m 5'-nucleotidase (reaction 4). Therefore, the formation of AMP from IMP is favored. Finally, high Pi stimulates ATP synthesis in mitochrondria by ATP synthase (reaction 1). The low Pi state reverses the conditions described. The relative lack of Pi decreases the resynthesis of ATP from ADP. Therefore, as ATP is consumed by reactions of intermediary metabolism and ADP is formed, low Pi favors the breakdown of ADP to uric acid. Low Pi levels lead to the activation of AMP deaminase (reaction 3) and high K_m 5'-nucleotidase (reaction 4). Thus AMP breakdown to inosine is favored. Decreased synthesis of PRPP (reaction 8) decreases hypoxanthine salvage and de novo purine synthesis. Thus high Pi stimulates ATP synthesis, while low Pi favors the degradation of ATP to purine end products.

phosphate diet; (*State b*) State a with intravenous phosphate supplementation 30 min before and for 3 h fructose infusion; (*State c*) A phosphate-repleted state produced by oral phosphate supplementation over several days.

Volunteers were given Alucaps (5.7 g/d of aluminum hydroxide) for 7 d before the first fructose infusion and admitted to the Clinical Research Center at the University of Michigan Medical Center. The subjects were started on a low purine, low phosphorus (300 to 400 mg/d) isocaloric diet 5 d before the first fructose infusion. 2 d before the first fructose infusion, they were given 25 μCi of [^{14}C]adenine contained in 1.6 μmol adenine. Only a single injection of radioactive adenine was given.

Fructose was infused on the fourth, sixth, and eighth days of hospitalization using a 20% fructose solution (0.5 g/kg) infused over a 5- to 7-min time interval (15). Transient abdominal cramping was a rare side effect of fructose infusion. Urine and blood samples were collected from 2 h before infusion to 3 h after infusion. Samples were analyzed for plasma phosphorus, creatinine, uric acid, hypoxanthine, xanthine, adenosine, and inosine as well as urinary radioactivity, phosphorus, creatinine, uric acid, hypoxanthine, xanthine, and inosine. The first fructose infusion 4 d after hospital admission occurred in a clinical setting of relative phosphorus depletion. On day 6 after admission, patients were infused with sodium phosphate (0.03 mmol/kg per h) starting 30 min before fructose infusion and continuing for 3 h after infusion. This represents an acute phosphorus repletion state. After intravenous phosphorus supplementation, patients were given a low purine, high phosphorus (3 g/d) diet and Neutraphos (4.6 g phosphate/d). Fructose infusion was performed 8 d after admission. This represents a phosphate-rich state.

Assays. Serum phosphorus was measured by University of Michigan Clinical Chemistry laboratory by the phosphomolybdate method. Plasma and urinary hypoxanthine, xanthine, inosine, and adenosine were measured by high pressure liquid chromatography as previously described (17, 18). Erythrocyte phosphate levels were measured as described using trichloroacetic acid precipitation and the method of Chen (19, 20). Creatinine was measured by a modified Jaffe reaction (21). Plasma and urine uric acid and oxypurines were measured using enzymatic spectrophotometric techniques (22, 23). Urinary radioactivity was measured by adding an 0.5-ml aliquot of each urine sample to 5 ml ACS scintillation fluid with samples counted in a liquid scintillation counter (1217 Racbeta; LKB Instruments, Inc.) (16). Trichloroacetic acid extracts of cell protein were solubilized with 0.5 N sodium hydroxide and the protein was measured by the method of Lowry (24).

Data analysis. Data were stored and analyzed using CLINFO PLUS on a microVax II minicomputer in the Clinical Research Center, University of Michigan Medical Center. Urinary purine specific activity was calculated by dividing total radioactivity values by total urinary purines (the sum of hypoxanthine, xanthine, inosine, and uric acid) (25). This specific activity is the measure that most closely reflects in vivo ATP pool specific activity.

The data were analyzed statistically with a repeated measures analysis of variance to determine the effect of Pi state (low phosphate, phosphate supplement, and high phosphate), the effect of time and interactions between Pi state and time. Multiple comparisons were performed using Fisher's least significant difference procedure. When necessary, the natural logarithms of the data were analyzed instead of the raw data to stabilize the variance. A $P < 0.05$ was considered significant. Variables are expressed as mean±1 SD.

Results

Baseline measurements. When baseline phosphorus values for states a and b were compared to state c, a statistically significant increase ($P < 0.01$) was seen in serum phosphorus and log urine phosphorus excretion (Table I). The significantly higher values in state c indicate that it represents a relative phosphate-repleted state. Baseline values in state b were obtained before phosphorus resupplementation and are analogous to state a. The baseline plasma and urinary purines, urinary radioactivity, and urinary purine specific activity were not altered by the changes in phosphate levels.

Measurements during ATP degradation. ATP degradation was produced by intravenous fructose. Plasma phosphorus rapidly declined after fructose infusion (Fig. 2). In all three study conditions, phosphorus values were 74–80% of baseline values by 15 min after fructose infusion. The overall serum phosphorus level was significantly higher in the "high phosphate state" than in the other two states ($P < 0.05$). Similarly, erythrocyte phosphorus content rapidly declined to 76–86% of baseline values within 15–30 min after fructose infusion. Although a higher erythrocyte phosphorus content was asso-

Table I. Clinical and Laboratory Characteristics of Study Patients

Patient No.	Age	Serum creatinine	Serum phosphorus			Urinary phosphorus excretion		
			a	b	c	a	b	c
	yr	*mg/dl*		*mg/dl*			*mg/g creatinine*	
1	24	0.9	3.8	3.7	4.6	38.4	126.02	285.7
2	25	1.0	3.1	4.3	4.3	54.8	63.3	543.6
3	23	1.0	4.3	3.7	5.6	98.8	105.3	525.4
4	22	1.1	3.7	4.1	4.5	246.2	295.9	508.9
5	23	1.2	3.6	3.5	5.0	84.1	160.2	819.7
Mean±SD	23.4±1.1	1.0±0.1	3.7±0.4	3.9±0.3	4.8±0.5*	104.±82.7	150.1±88.7	936.7±764.9‡

(*a*) Relative phosphate depletion. (*b*) Supplementation with intravenous phosphorus 30 min before fructose infusion. (*c*) Treated with neutraphos/high phosphorus diet for 2 d. * T = 4.7, $P = 0.009$; ‡ T = 4.5, $P = 0.011$.

ciated with a phosphate-repleted state, this difference was not statistically significant ($P = 0.25$). The latter resulted from substantial variability in the data and small changes.

Total plasma purines increased by 40–50% within 30 min after fructose infusion (Fig. 3). There was no significant difference in basal levels ($P = 0.057$) among the three states. However, there was a significant linear increase in levels after 30 min overall ($P = 0.0002$), such that the mean postinfusion level was significantly elevated over the mean basal level of plasma purines ($P = 0.001$). There were no significant differences between the three Pi states, either before infusion ($P = 0.09$) or after infusion ($P = 0.32$), nor were there any significant Pi state and time interactions.

Urinary purines significantly increased an average of fourfold in all three states of phosphorus intake at 60 min ($P = 0.0001$) from an average baseline value of 1.16 mmol/g creatinine, and then declined to a level significantly higher than baseline ($P = 0.001$). There were no significant differences among groups either at baseline, at 60, or after 60 min.

Urinary radioactivity excretion was fourfold higher in the phosphate-depleted state compared to the lower values in the relatively phosphate-repleted state at 60 min after infusion (Fig. 4). Differences between each of these states at 60 min were statistically significant (state a vs. c, $P < 0.001$; state a vs. b, $P < 0.01$; state b vs. c, $P < 0.05$). The urinary specific activity at 60 min was over threefold higher in the low-phosphate state (a) vs. the phosphate-repleted state (c) and twofold higher in the phosphate-supplemented state (b) vs. the phosphate-repleted state (c) (Fig. 5). All pairwise differences between the three phosphate states were statistically significant (state a vs. c, $P < 0.001$; state a vs. b, $P < 0.001$; state b vs. c, $P < 0.01$). The differences between the 60-min time point and the other time points were statistically significant ($P < 0.001$), while the differences among the remaining time points were not significant for either urinary radioactivity or urinary specific activity.

Discussion

Inorganic phosphate is a rate-limiting substrate for ATP synthesis in the mitochondrial sequence of oxidative phosphorylation (26). Whether phosphate concentration in vivo is rate

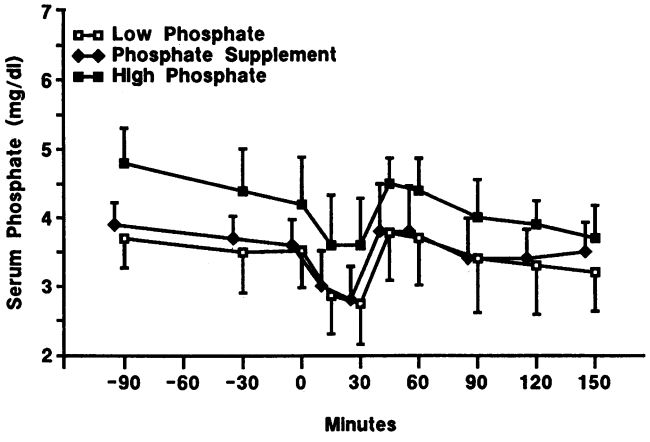

Figure 2. Effect of fructose infusion on serum phosphate levels. This graph demonstrates the changes in serum inorganic phosphate levels before, during, and after fructose infusions during relative phosphate depletion, phosphate infusion, and phosphate repletion. The data are presented as mean plus or minus the standard deviation in this figure and in all other figures.

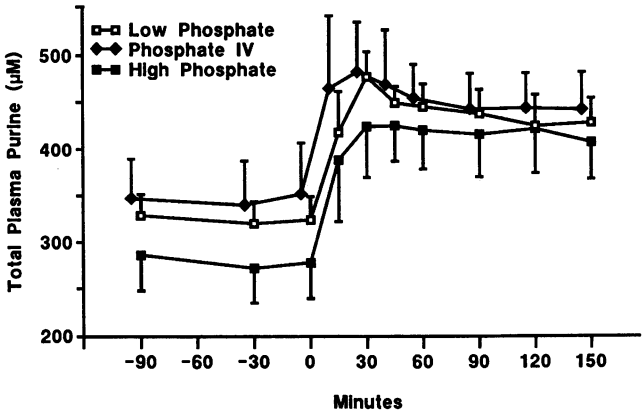

Figure 3. Effect of fructose infusion on total plasma purines. Total plasma purine includes the sum of hypoxanthine, xanthine, adenosine, inosine and uric acid. There is a marked increase in plasma purines with fructose infusion, but there is no difference in the increases between the phosphate-depleted and phosphate-repleted states.

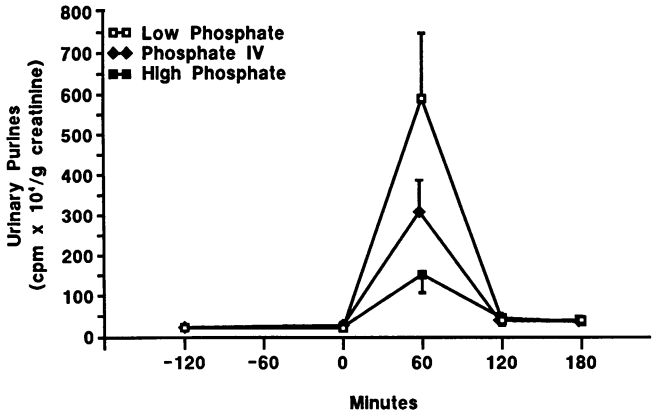

Figure 4. Effect of fructose infusion on urinary radioactive purine excretion. Although the baseline excretion of radioactively labeled urinary purines is the same in all three states, there are major differences in the rate of excretion of radioactivity. State a (low phosphate) has the highest rate of excretion of radioactivity followed by state b (the intravenously infused phosphate) and state c (high phosphate). Differences between each of these states were statistically significant (state a vs. c, $P < 0.001$; state a vs. b, $P < 0.01$; state b vs. c, $P < 0.05$).

limiting for ATP synthesis is an important issue with therapeutic implications. Such a possibility is suggested by the fact that the normal plasma phosphate concentration of 1 mM approximates the apparent K_m of phosphate for mitochondrial respiration (26). This is further supported by observations that altered organ function has been linked to decreased intracellular phosphate and associated reduced ATP levels. Severe hypophosphatemia < 1 mg/dl is associated with specific clinical disorders including respiratory failure, muscle weakness, hemolytic anemia, neurologic abnormalities, and altered renal tubular function (7–14, 27, 28). These disorders typically occur in clinical settings of hyperalimentation, treatment of diabetic ketoacidosis, alcohol withdrawal, and overzealous use of phosphate binding antacids. There is a correlation between hypophosphatemia and decline in erythrocyte ATP levels in

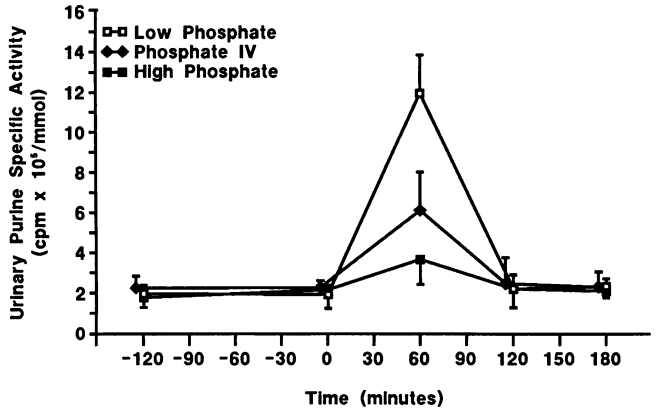

Figure 5. Effect of fructose infusion on urinary purine specific activity. The baseline urinary purine specific activity is identical for the three states of phosphorus depletion or repletion. The difference between the phosphate-depleted state and phosphate-supplemented state or the phosphate-repleted states were statistically significant (state a vs. state c, $P < 0.001$; state a vs. b, $P < 0.001$; state b vs. c, $P < 0.01$).

renal failure and intravenous hyperalimentation (10, 11). In hypophosphatemic mice skeletal muscle weakness and renal cortical changes have been associated with elevated levels of AMP, inosine, and hypoxanthine (5, 6, 27). Furthermore, a fructose-induced renal acidification defect in patients with hereditary fructose intolerance can be explained by fructose related depletion of inorganic phosphate and renal cortical ATP levels (28, 29).

In this study we have produced a relative hypophosphatemic state by the use of aluminum hydroxide tablets and dietary restriction (7, 30) and compared this with a state of relative phosphate repletion. Changes in plasma and urinary phosphorus levels have confirmed the efficacy of our experimental interventions. A state of rapid ATP degradation was induced by the use of fructose infusions (1–4, 15). This results from a diminution in intracellular phosphate levels secondary to the accumulation of fructose-1-phosphate and the reduced synthesis of ATP from ADP in mitochondria (1–4, 29). In our studies relative phosphate depletion causes a major increase in the excretion of radioactive purines as compared with the phosphate-repleted state. In addition, there was an increased specific activity of the radiolabeled purines in the urine of subjects with a relative phosphate depletion. These observations suggest that during relative phosphate depletion ATP degradation is greater than during phosphate repletion.

The degree of inorganic phosphate depletion determines the severity of the decrease in adenine nucleotide levels in other studies. In fructose-loaded rats there is a direct correlation between phosphate concentrations and adenine nucleotide levels (29). Prior phosphate loading of the rats largely prevented the reduction of ATP and total adenine nucleotides in the renal cortex and liver. In gouty patients pretreatment with a phosphate infusion prevented fructose induced hyperuricemia (31). Taken together, these observations support the concept that hypophosphatemia decreases ATP synthesis from ADP and thereby promotes ADP degradation to purine end products. Repletion of phosphate promotes ATP synthesis from ADP and decreases ADP degradation.

If there is accelerated ATP degradation to purine end products, why is the change in absolute levels of plasma and urinary purines not different with relative phosphate depletion as compared to phosphate repletion? The explanation for this similiarity lies in the existence of two distinct pathways that supply purine degradation products to the body fluids: the ATP degradation pathway and the pathway of de novo purine synthesis (Fig. 1). These events have been carefully examined in rat liver (32, 33) and explain the results of fructose infusion in humans. Under normal conditions fructose infusion first activates ATP degradation and then later activates de novo purine synthesis (32, 33). During the first 5 min after a fructose infusion there is marked ATP depletion, which is associated with intracellular phosphate depletion and reduction of phosphoribosylpyrophosphate (PRPP) levels. The hypophosphatemia releases the inhibition of AMP deaminase and high K_m 5'-nucleotidase (3) and the depletion of PRPP decreases de novo purine synthesis and hypoxanthine reutilization (32, 33). This facilitates the conversion of ATP degradation products to purine end products. At 15–30 min ATP depletion is partially reversed, inorganic phosphorus levels return to normal, and PRPP levels are markedly elevated (32, 33). PRPP synthetase, which synthesizes PRPP, is highly sensitive to activation by increasing inorganic phosphate levels (34). The marked in-

creases of PRPP levels at 15–30 min stimulate de novo purine synthesis and hypoxanthine reutilization (32, 33). Inhibition of AMP deaminase and high K_m 5′-nucleotidase are restored. Under these conditions metabolic regulation favors ATP resynthesis. Therefore, during the first 5 min of fructose infusion the degradation of ATP leads to the appearance of ATP breakdown products in body fluids. At 15–30 min the purines appearing in the urine and in the plasma mainly originate from intensive stimulation of de novo purine synthesis which forms the same purine end products, which are not radioactively labeled.

On the basis of these data we speculate upon the origin of urinary purines. ATP synthesis and de novo purine synthesis are both phosphate sensitive (Fig. 1). During a relative phosphorus depleted state the amount of ATP resynthesized from ADP during a fructose infusion will be diminished, since there is less phosphate substrate available for this conversion. Thus during hypophosphatemia more ATP will be degraded to purine end products. On the other hand, during hypophosphatemia at 15–30 min there will be less of an increase in the intracellular phosphate levels. This will limit the increase in PRPP levels and the increase in de novo purine synthesis. Therefore, there will be a decreased excretion of purines at 15–30 min originating from de novo purine synthesis. The net effect of hypophosphatemia compared to the normal phosphate state is an increased excretion of purines originating from ATP and a decreased excretion of purines originating from de novo purine synthesis. When the ATP pool is prelabeled with radioactive adenine, as in our system, this causes an enhanced enrichment of urinary purines by radioactivity. This concept is supported by our studies showing that the specific activity of the urinary purines in the phosphate-repleted state is lower than the phosphate-depleted state. This suggests that purines excreted in the phosphate-repleted state are originating from a source other than ATP degradation. This other source must be de novo purine synthesis.

Our studies are significant for a number of reasons. Firstly, they suggest that phosphate is a rate-limiting metabolite for ATP synthesis in vivo at least under conditions of a fructose infusion. Increased synthesis of ATP during relative phosphate repletion diminishes ATP degradation to purine endproducts. Secondly, the ability to stimulate ATP synthesis by inorganic phosphate therapy may have clinical relevance. Phosphate supplementation may help reverse potentially ATP related hypophosphatemic consequences of diabetic ketoacidosis, alcohol withdrawal, intestinal dysfunction, malnutrition, anorexia nervosa, or cancer (35). Similarly, the degradation of ATP in glycogen storage disease type I and hereditary fructose intolerance occurs by a mechanism similar to fructose induced hyperuricemia (1–4, 28, 29, 36, 37) and may be reversed by phosphorus loading. There is evidence that ATP degradation may occur as a consequence of hypoxia (1–4). Whether inorganic phosphorus supplementation together with oxygen repletion and other substrate administration can accelerate the resynthesis of ATP under these conditions remains unclear, since the main problem is oxygen lack. Finally, further experiments examining the regulation of ATP metabolism by phosphorus are necessary. The proposed major stimulation of ATP degradation in relative hypophosphatemia during the first 5 min of a fructose infusion, should be evaluated in clinical studies by examining ATP degradation in a period of minutes after fructose infusion rather than the longer time periods used in standard experiments. In any event the ability to regulate ATP metabolism in vivo by inorganic phosphorus levels may provide an innovative therapeutic approach to modify disordered energy metabolism in specific disease states (1–4, 35, 38).

Acknowledgments

The authors wish to thank the house officers, nurses, and dieticians of the University of Michigan Clinical Research Center for their excellent care of the study subjects, Sandy Ostrowski for measurement of plasma and urinary uric acid and creatinine and Trina Freeman, Paige Bigelow, and Jeanne Schmaltz for typing this manuscript.

Supported by grants from U. S. Public Health Service (2R01 DK-19674 and 5 M01 RR-42). Dr. Johnson is a postdoctoral fellow of the Rackham Arthritis Research Unit supported by U. S. Public Health Service training grant T32-AR07080.

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