Hypophosphatemia and Rhabdomyolysis

J. P. Knochel, C. Barcenas, J. R. Cotton, T. J. Fuller, R. Haller, and N. W. Carter, Veterans Administration Hospital, Dallas, Texas 75216 and The University of Texas Health Science Center at Dallas, Southwestern Medical School, Dallas, Texas 75235

ABSTRACT Clinical observations suggest that overt rhabdomyolysis may occur if severe hypophosphatemia is superimposed upon a pre-existing subclinical myopathy. To examine this possibility, a subclinical muscle cell injury was induced in 23 dogs by feeding them a phosphorus- and calorie-deficient diet until they lost 30% of their original weight. To induce acute, severe hypophosphatemia in the animals after partial starvation, 17 of the dogs were given large quantities of the same phosphate-deficient diet in conjunction with an oral carbohydrate supplement, which together provided 140 kcal/kg per day.

After phosphorus and caloric deprivation, serum phosphorus and creatine phosphokinase (CPK) activity were normal. Total muscle phosphorus content fell from 28.0±1.3 to 26.1±2.5 mmol/dg fat-free dry solids. Sodium, chloride, and water contents rose. These changes resembled those observed in patients with subclinical alcoholic myopathy. When studied after 3 days of hyperalimentation, the animals not receiving phosphorus showed weakness, tremulousness, and in some cases, seizures. Serum phosphorus fell, the average lowest value was 0.8 mg/dl (P < 0.001). CPK activity rose from 66±357 to 695±1,288 IU/liter (P < 0.001). Muscle phosphorus content fell further to 21.1±7.7 mmol/dg fat-free dry solids (P < 0.001). Muscle Na and Cl contents became higher (P < 0.01). Sections of gracilis muscle showed frank rhabdomyolysis.

6 of the 23 phosphorus- and calorie-deprived dogs were also given 140 kcal/kg per day but in addition, each received 147 mmol of elemental phosphorus. These dogs consumed their diet avidly and displayed no symptoms. They did not become hypophosphatemic, their CPK remained normal, and derangements of cellular Na, Cl, and H2O were rapidly corrected. The gracilis muscle appeared normal histologically in these animals.

These data suggest that a subclinical myopathy may set the stage for rhabdomyolysis if acute, severe hypophosphatemia is superimposed. Neither acute hypophosphatemia nor rhabdomyolysis occur if abundant phosphorus is provided during hyperalimentation.

INTRODUCTION

Recent observations have shown that most severe alcoholics with either clinical or laboratory evidence of alcoholic myopathy have abnormally low total phosphorus content in skeletal muscle (1). Many of these patients demonstrate normal or slightly subnormal values for serum phosphorus concentration when they are admitted to the hospital. However, during the first few hospital days, hypophosphatemia appears and may become progressively more severe. It generally occurs as nutrients are administered and from all evidence, results from phosphorus movement into cells (2). At least in some patients, this decline of serum phosphorus is associated with a sharp rise of serum creatine phosphokinase (CPK) activity (1), suggesting that severe hypophosphatemia may be responsible for converting a subclinical myopathy into acute rhabdomyolysis.

In previous reports, we have shown an abnormally low total phosphorus content of muscle tissue in patients with alcoholic myopathy. They also demonstrated elevated muscle content of sodium, chloride, and water. Directly measured transmembrane electrical potential difference of skeletal muscle cells was abnormally low (1). Such abnormalities suggest a defect in ion transport. Subsequent studies in dogs (3) showed that experimental phosphorus deficiency led to weakness, anorexia, and an electrochemical disturbance of muscle cells closely resembling that observed in patients with alcoholic myopathy. This was reversible upon phosphorus repletion. Of interest, hypophosphatemia developed gradually and did not become severe in these experimental animals. In addition, CPK activity re-

1Abbreviations used in this paper: CPK, creatine phosphokinase; FFDS, fat-free dry solids.

---

Received for publication 12 May 1978 and in revised form 17 July 1978.
mained within normal limits despite chemical derange-
ments of the muscle cells. These features were quite
similar to our observations on patients with alcoholic
myopathy before they developed severe hypophospha-
temia (1). Therefore, whereas simple phosphorus de-
iciency may lead to a subclinical cellular injury, the
development of acute rhabdomyolysis may require
superimposition of an additional insult, such as hy-
ppophosphatemia.

The purpose of this study was to determine if hy-
ppophosphatemia induced by hyperalimentation can
produce acute rhabdomyolysis in the dog if it is super-
imposed upon an existing electrochemical injury. A
subclinical myopathy was induced by phosphorus de-
privation and partial starvation so that severe hypo-
phosphatemia would likely occur upon re-feeding
without phosphorus. The results show that administra-
tion of excessive calories to an animal prepared in this
manner causes severe hypophosphatemia and acute
rhabdomyolysis. Such hyperalimentation in the same
animal preparation causes neither hypophosphatemia
nor rhabdomyolysis if adequate elemental phosphorus
is provided in the diet.

METHODS

The dog model used in these studies was developed to stimu-
late certain features of severe, chronic alcoholics found to
have myopathy. These patients commonly have lost body
weight and are phosphorus deficient. 23 healthy male mongrel dogs weighing from 22 to 25 kg were each studied on three occasions: (a) control, (b) after weight loss and phosphorus deprivation, and (c) after hyperalimentation with and without supplemental phosphorus. Control studies were conducted after the animals had received a synthetic, phosphorus-deficient but otherwise nutritionally adequate diet for 7 days. Before the control study, 1.87 g of Na3HPO4 was added to the diet each day. The diet was obtained from ICN Pharmaceuticals Inc., Life Sciences Group, Cleveland, Ohio. Each 100 g contained 410 calories, 20 g of protein, 60 g of carbohydrate, and 10 g of fat. Upon chemical analysis, each 100 g of diet contained 26 mg of ele-
mental phosphorus, 3 mmol of magnesium, and 36 meq of potassium. In other respects the diet contained all vitamins, minerals, and electrolytes in excess of minimum require-
ments. For the control study, sufficient diet was fed to pro-
vide 30 calories/kg per day. On day 8, when the dogs were
under sedation with 15 mg/kg body wt pentobarbital, serum
CPK, plasma and muscle sodium, potassium, chloride, and
total phosphorus were measured. Venous blood was also
sampled for pH, carbon dioxide tension, and oxygen tension.
For determination of composition, samples of the gracilis
muscle were collected with a needle biopsy instrument and
analyzed as previously described (3, 4).

After the control study, the dogs were placed on the same
synthetic diet without added phosphorus but in a reduced
quantity to provide 15 calories/kg per day. Aluminum carbon-
ate gel (Basalgel, Wyeth Laboratories, Philadelphia, Pa.),
60 ml, was mixed into the diet each day to bind phosphorus
in the gut. The dogs were weighed daily before feeding.
The second study, identical to the first, was conducted when the
animals had lost 30% of their initial body weight.

After the effects of weight loss and phosphorus deprivation
were determined, 17 of the animals were begun on 500 g
of the same diet without added phosphorus but with suffi-
cient added carbohydrate to provide 140 calories/kg per day.
The extra carbohydrate was provided as a solution that con-
tained 10 g glucose and 40 g of maltose/100 cm³. One-half
of the diet was given in the morning and one-half in the after-
noon. Any diet not consumed spontaneously was fed by va-
gage. The remaining six dogs were fed the same diet without
aluminum carbonate. This diet contained phosphorus and pro-
vided 140 calories/kg per day. Because both hypophospha-
temia and hypokalemia may occur under conditions of such
hyperalimentation, sodium and potassium phosphate were ad-
ministered in the following proportions: 3 mmol Na2HPO4,
8 mmol NaH2PO4, 3 mmol K2HPO4, and 3.2 mmol KH2PO4. 20 g
of this mixture was given to each dog daily. This quantity con-
tained 147 mmol of phosphorus and 79 meq of potassium.
This quantity contained 147 mmol of phosphorus and 79 meq
of potassium. Fasting blood samples were collected on each
morning during hyperalimentation. Skeletal muscle was ob-
tained 72 h after initiation of hyperalimentation unless the
animal appeared seriously ill before that time. For the same
reason, some dogs were biopsied on the 2nd or 3rd day of
hyperalimentation. For examination of muscle histology,
stretched sections of gracilis muscle were removed, fixed in
buffered formalin, and stained with hematoxylin and cosin.

Serum sodium, potassium, and chloride determinations
were performed by conventional laboratory procedures.
Serum phosphorus was measured by the colorimetric method
used for the Technicon AutoAnalyzer (Technicon Instru-
ments Corp., Tarrytown, N. Y.). Muscle sodium, chloride, and
potassium contents were determined on dilute acetic acid ex-
tracts of dried, fat-extracted samples that weighed 10–20 mg
as previously described (4). Serum CPK activity was meas-
ured spectrophotometrically by a modification of the Rosalki
procedure (5). After weight loss and hyperalimentation elec-
trolytes and total phosphorus in muscle were compared indi-
vidually to their respective values obtained during the con-
tral study by the paired t test. All data are expressed as
mean ± 1 SD.

RESULTS

As noted previously, dogs fed a phosphorus-deficient
diet generally became anorectic and had to be gavage fed
when the diet was not voluntarily consumed. Ex-
cept for equivocal weakness and diminished spontane-
ous physical activity, the animals displayed no unusual
symptoms during the period of caloric depriva-
tion. From 28 to 40 days were required for the dogs to
lose 30% of their initial weight. During the period of
hyperalimentation those not given a phosphorus sup-
plement became extremely ill. They demonstrated trem-
ulousness, difficulty in standing, and severe weak-
ness. Some displayed convulsive movements. Four died
by the 3rd day of hyperalimentation. Data on these
animals were excluded.

Individual values for serum phosphorus concen-
tration are shown in Fig. 1. The average value in the
control study was 4.4 ± 0.8 mg/dl. After the dogs re-
ceived the phosphorus-deficient diet and lost 30% of
their original body weight, the serum phosphorus was
3.9 ± 1.2 mg/dl. This was not significantly different from
the control value. After 3 days of hyperalimentation,
FIGURE 1 Serum phosphorus concentration before and after phosphorus deprivation and starvation (PD-S) and after hyperalimentation without supplemental phosphorus.

The average value was 1.2 ± 1.3 mg/dl (P < 0.001). The average lowest value observed during the 3 days of hyperalimentation was 0.8 ± 0.6 mg/dl. One animal showed a serum phosphorus of 5.6 mg/dl. This dog was extremely ill and before death had a serum urea nitrogen above 100 mg/dl. Its urine showed heme positive pigment which was presumably myoglobin.

Serum CPK activity is shown in Fig. 2. Before caloric deprivation, CPK averaged 52 ± 22 IU/ml. After weight loss and the phosphorus-deficient diet, CPK activity averaged 66 ± 357 IU/ml. These values were not significantly different. However, hyperalimentation without supplemental phosphorus was associated with a broad range of elevated CPK values in 14 of 17 animals. The average highest value was 695 ± 1,288 IU/ml. The isolated value of 5,470 IU/ml was observed in the same dog that showed hyperphosphatemia, azotemia, and evidence of myoglobinuria. Kidney tissue was not examined.

Total phosphorus content of skeletal muscle is shown in Fig. 3. In the control period, total phosphorus averaged 28.0 ± 1.3 mmol/100 g fat-free dry solids (FFDS). After the caloric-deficient diet without phosphorus, average muscle phosphorus was 26.1 ± 2.5 mmol/dg FFDS. This value was significantly different from control (P < 0.01). Two animals showed a normal value for muscle phosphorus content. After hyperalimentation, muscle phosphorus declined moderately and averaged 21.1 ± 7.7 mmol/dg FFDS (P < 0.001).

Muscle potassium, sodium, chloride, and water are shown in Fig. 4. Potassium content averaged 44.1 ± 2.4 meq/dg FFDS in the control study. After weight loss and phosphorus deprivation, potassium averaged 44.6 ± 1.9 meq/dg FFDS. After hyperalimentation, muscle potassium fell slightly to 40.3 ± 4.9 meq/dg FFDS (P < 0.01). Hypokalemia did not occur. Muscle sodium content rose from 10.2 ± 1.1 to 13.5 ± 4.2 meq/dg FFDS after weight loss and phosphorus deprivation. After hyperalimentation, sodium rose further to 16.1 ± 9.9 meq/dg FFDS. This value was significantly higher than the normal range.
different from control \(P < 0.05\) but not different from
the value measured after caloric deprivation. After
calic and phosphorus deprivation, muscle chloride
content rose from 8.3\(\pm\)9.3 to 11.9\(\pm\)3.9 meq/dg FFDS.
This was not significantly different from that deter-
m ined in the control period. However, after hyperali-
mentation, chloride rose further and averaged 14.1\(\pm\)8.4
meq/dg FFDS. This was significantly different from
the control value \((P < 0.001)\) but not from that estab-
lished after caloric deprivation. Muscle water content
rose from 351\(\pm\)27 to 380\(\pm\)34 ml/dg FFDS after caloric
deparation. After hyperalimentation, water remained
essentially unchanged at 379\(\pm\)26 ml/dg FFDS. This
was also significantly different from control \((P < 0.05)\)
but not significantly different from that determined
after caloric deprivation.

In sharp contrast to dogs not given a phosphorus
supplement, those that received a phosphorus supple-
ment during hyperalimentation avidly consumed their
diets and appeared healthy. Despite an intake of 147
mmol of elemental phosphorus daily, there was no
diarrhea. Hypophosphatemia did not occur (Fig. 5).
In these dogs, average serum phosphorus after 3 days
of hyperalimentation was 3.6\(\pm\)0.7 mg/dl. This is
markedly different from the lowest average of 0.8\(\pm\)0.6
mg/dl in the dogs not receiving phosphorus supple-
ments during hyperalimentation.

Muscle phosphorus content after 3 days of hyperalimen-
tation and phosphorus supplementation was 25.3\(\pm\)3.3
mmol/dg FFDS. This value was not significantly dif-
ferent from control. CPK activity also remained normal.
Sodium, chloride, and water content of skeletal muscle
in the six dogs hyperalimented and supplemented with
phosphorus rapidly returned to normal (Fig. 4). These
events stand in contrast to the climbing sodium and
chloride content of muscle in dogs hyperalimented
without supplemental phosphorus. Similar to animals
re-fed without phosphorus, muscle potassium re-
mained slightly below the control value, 39.7\(\pm\)3.7
meq/dl FFDS. This was significantly different from
control \((P < 0.05)\) but not different from the dogs re-
fed without phosphorus. Surprisingly, the slightly low
potassium content in skeletal muscle of the dogs re-fed
with the phosphorus supplement persisted despite an
average potassium intake of 250 meq/day. Histologi-
cally, skeletal muscle showed gross rhabdomyolysis in
partially starved dogs hyperalimented without phos-
phorus (Fig. 6). In those dogs hyperalimented with sup-
plemental phosphorus, the muscle appeared normal
(Fig. 7).

**DISCUSSION**

These studies show that phosphorus deprivation and
partial starvation induce a subtle chemical injury of
muscle cells characterized by a decline of total phos-
phorus and elevated content of sodium, chloride; and
water. These findings are similar to those we have ob-
served in chronic, severe alcoholics (1). Under both
sets of circumstances, serum phosphorus concen-
tration is generally normal or only moderately depressed
and serum CPK activity is usually normal or only slightly
elevated. In either severe alcoholics or dogs as pre-
pared in these studies, administration of nutrients leads
to hypophosphatemia. This is often followed by an
abrupt rise of CPK activity. As illustrated by these
studies, the rise of CPK activity is associated with his-
tologic evidence of frank rhabdomyolysis.

The experimental model employed in these studies
resembles acute hypophosphatemic rhabdomyolysis in
man. Our observations suggest that hypophosphate-
temia per se and not hyperalimentation triggered rhab-
domyolysis. This is supported by the observation that
providing enough phosphorus in the diet to prevent
hypophosphatemia during identical hyperalimentation
prevents rhabdomyolysis. Indeed, adequate phos-
phorus supplementation during hyperalimentation
rapidly corrected abnormal accumulations of sodium,
chloride, and water in muscle tissue. In these hyper-
alimented, phosphorus-supplemented animals, the
skeletal muscle appeared normal by light microscopy.
The apparent inverse relationship between hypophos-
phatemia and elevated CPK activity is shown in Fig. 8.

**Figure 5** Serum phosphorus, muscle phosphorus, and
serum CPK activity before and after phosphorus depriva-
tion and starvation (PD-S) and after hyperalimentation with sup-
plemental phosphorus.
The light microscopic appearance of gracilis muscle from a partially starved dog hyperalimented without phosphorus. The muscle fibers are grossly distorted, hemorrhagic, and infiltrated by round cells and polymorphonuclear leukocytes. Some residual cross-striations remain in the center fiber (×600).

Plotted in this manner, the data suggest that CPK activity becomes elevated only when serum phosphorus falls below 1.5-2.0 mg/dl.

In addition to hypophosphatemia, phosphorus deficiency in muscle could have also contributed to the precipitation of acute rhabdomyolysis. In our previous studies of phosphorus-deficient dogs in which CPK was normal, total muscle phosphorus averaged 22.4 mmol/dg FFDS. In our current studies, muscle phosphorus averaged 21.1 mmol/dg FFDS. Although these values are nearly the same, it is possible that the rate at which muscle phosphorus fell in the hyperalimented dogs might have been an important factor underlying the sudden appearance of rhabdomyolysis.

The biochemical basis for cellular injury in hypophosphatemia may be severe depletion of ATP content (6). When phosphorus supplies are inadequate in the face of rapid cellular phosphorus uptake, hypophosphatemia results. Because inorganic phosphorus is in diffusion equilibrium across the plasma membrane (7), intracellular phosphorus would also fall. Abundant evidence suggests that a severe decline of inorganic phosphorus inside the cell interferes with regeneration of ATP from ADP. In turn, a low concentration of intracellular inorganic phosphorus activates AMP deaminase and the decline of ATP activates 5'-nucleotidase. The result is a critical loss of adenylic acids and in effect, an elimination of energy supplies within the cell. Such events have been reported in the brain (8), leukocytes (9), platelets, and erythrocytes (10). Whether or not such phenomena occur in muscle cells as a result of hypophosphatemia and precede rhabdomyolysis has not been examined.

Physical exertion could have also played a role in rhabdomyolysis in these dogs. Thus, tremors and, in some instances, convulsive movements were common in severely hypophosphatemic animals. That physical activity and especially convulsive seizures may produce rhabdomyolysis and myoglobinuria is widely known (11). Moreover, they are especially apt to cause
rhabdomyolysis if a myopathy already exists. Acute hypophosphatemia has been shown to cause convulsive seizures in both men (12) and experimental animals (8). In most instances hypophosphatemia had developed over the course of 1–10 days as nutrients were administered to either man or animals that previously had lost body weight (9, 10). Although comparably severe hypophosphatemia can be readily induced by respiratory alkalosis (13), to our knowledge this of itself has not been associated with acute rhabdomyolysis.

Potassium deficiency can also cause rhabdomyolysis (14, 15). Muscle potassium content falls slightly in otherwise well nourished phosphorus-deficient dogs (3). As demonstrated in these studies, it also remained in the lower range of normal in undernourished, phosphorus-deprived dogs after hyperalimentation with or without phosphorus supplements. However, hypokalemia did not occur and the decline of muscle potassium content was not severe. Based upon experimental (14) and clinical (15) observations, such a slight fall in muscle potassium would not ordinarily cause rhabdomyolysis.

ACKNOWLEDGMENTS

The authors express their gratitude to Donna Brese, James Long, D. L. Morris, and Patti Line for their help in this project.
REFERENCES


