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Basic Research

Changes in Phosphorus Distribution During Total Parenteral Nutrition

Y. MASHIMA, M.D., M. OGAWA, M.D., Y. AOKI, M.D., N. ICHIKI, M.D., AND D. K. SWAN, M.D.

From The First Surgical Department, Chiba University School of Medicine, Chiba City, Japan

ABSTRACT. To investigate the mechanism of hypophosphatemia during total parenteral nutrition (TPN), changes in phosphate (P) contents in the liver and muscle of rats supported by TPN for 2 days at 270 cal/g were studied in 39 Sprague Dawley rats (200 g body weight), divided into 5 groups as follows: G-I: starved for 24 hr (n = 7); G-II: TPN (5 mEq P/1000 cal) after 24 hr starvation (n = 7); G-III: starved for 4 days (n = 7); G-IV: TPN (5 mEq P/1000 cal) after 4 days starvation (n = 9); G-V: TPN (35 mEq P/1000 cal) after 4 days starvation (n = 9). P contents of the tissues were measured colorimetrically. Results indicated that muscle P content decreased in the depleted rat supported by TPN with low P intake, while an increase in P content in the liver was a constant finding in each TPN group. Increase in P intake tended to preserve the P content in the muscle. These findings suggest that the changes in P distribution in major body tissues must be considered in addition to the changes in the serum inorganic P levels to evaluate an adequate P requirement for TPN.

It has been well recognized that total parenteral nutrition (TPN) with inadequate phosphorus (P) intake induces hypophosphatemia, which is accompanied by clinical manifestations such as confusion, weakness, anorexia, malaise, paresthesia, coma, or death.[1–5] Redistribution of P within the body has been assumed to be a cause of this decrease in serum phosphate.[6] Hill et al[7] indicated by isotope study that hypophosphatemia is the result of an increase in turnover of P of skeletal muscle. Derr and Zieve,[8] also by isotope study, suggested that hypophosphatemia is caused by the shift of P into bone, liver, and other parts of the carcass.

In this study, estimations of the P content in the liver and skeletal muscle of the rats supported by TPN were performed as an attempt to investigate further mechanisms causing hypophosphatemia during TPN.

MATERIALS AND METHODS

Thirty-nine Sprague Dawley rats weighing approximately 200 g were placed in 5 groups. Group I (G-II, or TPN, n = 7) rats were supported by TPN for 2 days at 270 nonprotein cal/kg/day after 24-hr starvation. Group III (G-III, or depleted, n = 7) rats were starved for 4 days. Group IV (G-IV, or TPN on the depleted with low P intake, n = 9) rats were on TPN (270 nonprotein cal/kg/day) with low P intake (5 mEq P/1000 cal) after 4 days starvation. Group V (G-V, or TPN on the depleted with high P intake, n = 9) rats were on TPN with the same calories as G-IV and a higher P intake (35 mEq/1000 cal) after 4 days starvation.

Water was given freely to each animal. One unit TPN solution contained 250 g glucose, 25 g amino acid (Ispole, Daigo Eiyo Co), 65 mEq sodium, 40 mEq magnesium, 35 mEq calcium, 5 or 35 mEq phosphate as indicated (1 mmol = 2 mEq), and 2.5 ml MVI in 1055 ml. Total calories were 1020 cal/unit. The feeding system developed by Steiger et al[9] was applied for TPN on the rats.

Infusion was started after insertion of the central venous catheter via the extrajugular vein. An animal was sacrificed after each treatment by decapitation. Immediately after blood was collected in a heparinized vessel, the whole liver was dissected out and weighed. Approximately 1 g fresh liver was weighed and homogenized with 10 ml ice-cold 5% trichloroacetic acid (TCA) solution and centrifuged. The supernatant was analyzed for acid-soluble inorganic phosphate and acid-soluble total P, according to methods to be described. The rest of the liver was kept frozen at −20°C for total liver P measurement. The head, neck, paws, and tail of the animals were excluded from the study. The remaining carcass was skinned and other organs were dissected out. The rest of the carcass, consisting of bone and muscle, was steamed for 1 hr at 1 kg/m^2, and the muscle was separated from bone by dissection.[10] According to preliminary study, the muscle weight from the excluded part of the body was <10% of the total muscle weight. Inorganic phosphate in the serum and the supernatant of the liver homogenate was measured by the method of Taussky and Shorr.[11]

Total liver P in the liver homogenate or acid-soluble total P was determined as follows: 1 ml sample (liver homogenate with TCA or the supernatant of this homogenate) was oxidized in a test tube by boiling for 30 min with 0.4 ml 76% sulfuric acid and 2 ml 2 N sodium permanganate. The inorganic phosphate thus produced was measured colorimetrically by addition of the solution (2 ml) containing sodium sulfite (2 N) and ammonium molybdate (2%), then 0.5 ml 2% hydroquinone. After subtracting the acid-soluble total P from the total liver

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P content, the part remaining was described as acid-insoluble P present in the liver. Organic P content in the acid-soluble fraction was calculated as the difference of total P in the acid-soluble fraction minus inorganic P in the same fraction. Whole muscle was minched thoroughly, an aliquot (about 1 g) was homogenized with 10 ml deionized water, then the total muscle P was measured by the same method applied for the total liver P.

The P content was expressed in mg P/g wet weight tissue, or in mg P/kg body weight of the rat before each treatment. The changes in P content were expressed as the percentage of the mean value of the control group. The Student t test was applied to evaluate the differences between the groups.

RESULTS

Body weight decreased slightly after 24-hr starvation (7% in G-I), increased slightly after TPN (4% in G-II), decreased markedly after depletion (24~28% in G-III, G-IV, and G-V), and regained slightly after 2 days TPN in the depleted rats (G-IV and G-V, Fig. 1).

The weight of the liver increased by 23% in G-II and decreased by 34% in G-III. The liver weight of the depleted rats recovered to the level before starvation after 2 days TPN. The weight of the muscle did not show appreciable change in G-III, decreased by 17% in the depleted rats, but recovered slightly after 2 days TPN (Table I, Fig. 1).

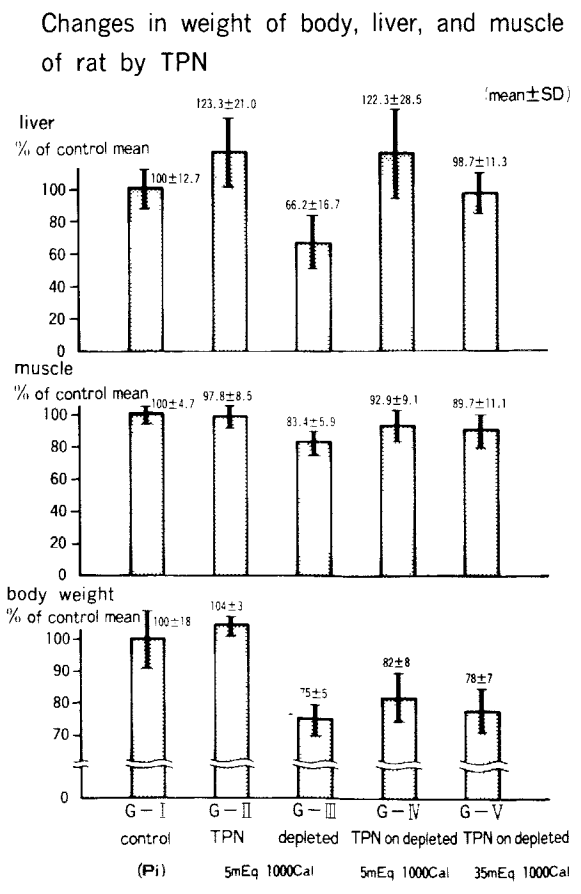

FIG. 1

TABLE I
Changes in weights and P contents of rat liver and muscle by TPN

	Liver						Muscle		
	Weight		P (mg/kg body weight)				Weight		Total P (mg/kg body weight) (% control)
	% Body weight	% Control	Soluble Pi	Soluble Po	Solid P	Total P (% control)	% Body weight	% Control	
G—I (control)	3.62 ± 0.46	100 ± 12.7	12.1 ± 2.1	19.2 ± 3.2	99.1 ± 13.6	130.4 ± 17.5 (100 ± 13.0)	30.9 ± 1.5	100 ± 4.7	609.1 ± 52.3 (100 ± 8.6)
G—II (TPN)	4.46 ± 0.76	123.3 ± 21.0	16.6 ± 4.7	24.6 ± 4.2	99.1 ± 15.7	140.2 ± 23.0 (107.5 ± 17.6)	30.2 ± 2.6	97.8 ± 8.5	568.0 ± 30.5 (93.3 ± 5.0)
G—III (starved)	2.40 ± 0.21	66.2 ± 16.7	7.6 ± 1.3	13.9 ± 2.1	65.1 ± 15.2	86.6 ± 17.5 (66.4 ± 13.4)	25.8 ± 1.8	83.4 ± 5.9	514.4 ± 54.8 (84.5 ± 9.0)
G—IV (TPN after starved c low Pin)	4.43 ± 1.03	122.3 ± 28.5	11.6 ± 2.0	27.5 ± 9.3	88.5 ± 12.1	127.7 ± 18.6 (97.9 ± 14.3)	28.7 ± 2.8	92.9 ± 9.1	446.3 ± 36.7 (73.2 ± 6.0)
G—V (TPN after starved c high Pin)	3.57 ± 0.41	98.7 ± 11.3	9.9 ± 1.4	21.8 ± 2.4	92.8 ± 15.5	123.9 ± 16.8 (95.0 ± 12.9)	27.7 ± 3.4	89.7 ± 11.1	499.6 ± 92.0 (82.0 ± 15.1)

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A preliminary experiment showed that cumulative urinary loss of P during 4 days starvation was 50.5 ± 0.6 mg (n = 4).

The serum inorganic phosphate (sPi) level was 9.2 ± 0.7 mg/dl in G-I, slightly decreased in G-II (8.1 ± 1.0 mg/dl) and G-III (7.7 ± 1.5 mg/dl). It was markedly decreased in G-IV where TPN with low P intake was performed on depleted animals. This decrement in sPi tended to be reduced by TPN with high P intake (Fig. 2).

Total liver P increased slightly but not significantly in G-II (TPN after 24-hr starvation, Fig. 2). When a parts/g tissue concentration of P was compared in G-I and G-III the results yielded no significant difference (Table II), but total liver P decreased by 34% in G-III because of the decrease in the size of the liver in G-III ($p < 0.001$, Table I, Fig. 2). TPN on the depleted rats resulted in a decrease in P concentration in the liver tissue (Table II), but owing to the increase in the liver size, total P content increased to the control level (Table I, Fig. 2). Although P intake was different, there was no significant difference in liver P content between G-IV and G-V (Fig. 2). In the muscle, total muscle P decreased to 84.5% of the control in G-III, and further decreased in G-IV. This decrease in muscle P tended to be prevented by higher P intake (G-V, Figure 2).

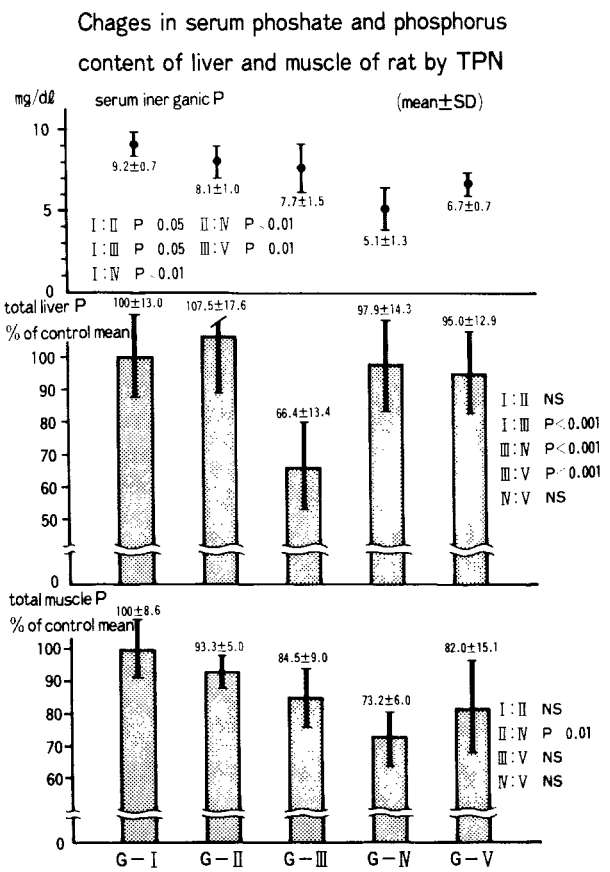

FIG. 2

TABLE II
Phosphorus contents in rat liver and muscle

	Contents	Liver	Muscle
		mg/g	
G—I	Control	3.61b ± 0.15	1.98 ± 0.17
G—II	TPN[a]	3.18 ± 0.41	1.87 ± 0.10
G—III	Depleted	3.60 ± 0.64	2.01 ± 0.22
G—IV	TPN on depleted[a]	2.97 ± 0.55	1.56 ± 0.16
G—V	TPN on depleted[b]	3.32 ± 0.36	1.79 ± 0.17

[a] P: 5 mEq/1000 Cal.
[b] P: 35 mEq/1000 Cal.

Liver P was separated into 3 fractions as shown in Table I and Figure 3. In the acid-soluble fraction, mean inorganic P (Pi) fraction was 12.1 mg/kg body weight, increasing to 16.6 mg/kg body weight in G-II. The total value of the acid-soluble Pi fraction decreased in G-III and increased in G-IV and G-V. Acid-soluble organic P fraction, representing high energy P such as ATP or sugar esters, increased in G-II, decreased by 30% in G-III, and increased in G-IV and G-V almost to the level of the control group. The acid-insoluble fraction, which exists mainly as phospholipid and nucleoprotein and constitutes 76% of total liver P in G-I, did not change in G-II and decreased in the depleted rat to 66% of the control. However, this decrease in acid-soluble P fraction in the depleted rat tended to increase toward the control level, by TPN with low or high P intake (G-IV, G-V). Thus, it can be said that all 3 P fractions decreased by long-term starvation, and more easily reversed at the same degree by TPN, whether P intake was high or low.

DISCUSSION

This study has shown that the total liver and muscle P content in the rat decreased to 66.4 and 84.5%, respectively, after 4 days starvation. During this period of

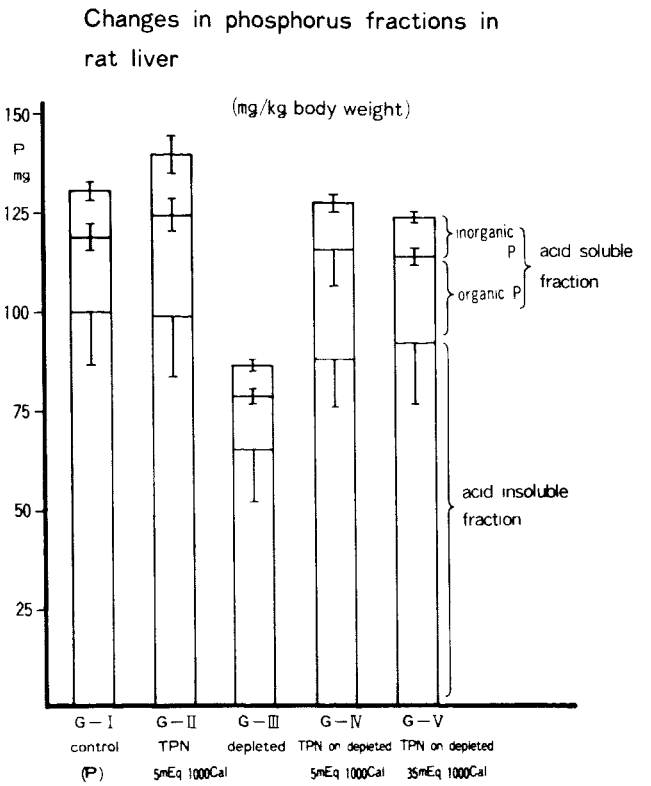

FIG. 3

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starvation, the animal lost 50 mg P from its urine. This amount of urinary loss is almost equivalent to twice as much as the total liver P content, or 40% of the total muscle content of the control rat. Administration of TPN on depleted rats resulted in an increase in liver P regardless of whether the animal was supplied with a low or high dose of P. This increment in liver P was approximately 40 mg/kg body weight, which is almost equal to 2 to 3 times more than the estimated extracellular inorganic phosphate (approximately 15 mg P/kg body weight, when extracellular inorganic P is tentatively calculated by assuming that 20% of the rat body weight is equivalent to extracellular space and that inorganic phosphate concentration in this space is the same level as that of serum inorganic phosphate in the depleted rat).

This increased P in the liver must be supplied from other body compartments if adequate P supply is not available. Although 80% of the body P is stored in bone and teeth,[10] the endogenous supply of P into the liver might have been slow or insufficient and other organs must have also been deprived of their P component to some degree as well. Hypophosphatemia may be the result of the appropriation of serum phosphate to the liver when it is induced to deposit P by TPN.

Muscle stores 9% of total body P[10] and any small change in muscle P concentration may influence total P content in the muscle. It thus has been supposed that muscle is one of the main tissues to take in P from other parts of the body, but this study indicates muscle behaves like blood, losing P with inadequate P supply during the initiation period of TPN. Further study on changes in P content in other vital organs might contribute to elucidate the finer mechanisms of hypophosphatemia.

Clinical manifestations accompanying TPN-induced hypophosphatemia have been assumed to be the result of hypoxia of peripheral organs induced by the increased affinity of the hemoglobin to the oxygen.[1] Disturbed glycolysis and the resulting decrease in red cell 2-3-DPG or ATP by a lack of an adequate amount of inorganic phosphate is the cause of this increase in oxygen affinity.[1, 2] As the same kind of disturbances in glycolysis or in production of high energy phosphate, such as ATP, should occur by a decrease in the cellular inorganic phosphate fraction, this might be another contributing factor in the development of the phosphate-depletion syndrome.[6]

In clinical TPN, however, changes in sPi levels and P balance are, at present, the practical means of assessing the subclinical deficiencies in patients. Accumulation of these data from TPN patients under differing clinical circumstances with different levels of P intake might be necessary to elucidate the adequate P intake during TPN, which at present are recommended at widely varying levels.[3, 12]

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