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Increased Metabolic Rate in X-Linked Hypophosphatemic Mice*

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ABSTRACT. *Hyp* mice are a model for human X-linked hypophosphatemia, the most common form of vitamin D-resistant rickets. It has previously been observed that *Hyp* mice have a greater food consumption per gram body weight than do normal mice. This led to the search for some alteration in metabolism in *Hyp* mice. We found that oxygen consumption was significantly higher in *Hyp* mice than in normal C57BL/6J mice and this was accompanied by an increased percentage of cardiac output being delivered to organs of heat production (liver and skeletal muscle), to the skin, and to bone and a decreased percentage to the gastrointestinal tract of *Hyp* mice. The increased oxygen consumption in *Hyp* mice was not associated with increased plasma free T_4 levels and was not affected by alterations in plasma phosphate produced by a low phosphate diet. The cause of the increased oxygen consumption is not known, and the role that this change and reported changes in distribution of cardiac output may play in the development of X-linked hypophosphatemia is also unknown. Study of the cardiovascular and thermoregulatory systems in *Hyp* mice should help increase understanding of the underlying mechanisms of this disease. (*Endocrinology* **118:** 441–445, 1986)

X-LINKED hypophosphatemia is an X-linked genetic bone disease characterized by reduced renal reabsorption of phosphate, hypophosphatemia, decreased growth rate and shortened stature, rickets, and osteomalacia (1). An animal model for this condition was described in 1976 by Eicher *et al.* (2). They described mice (known as *Hyp* mice) with a mutation on the X-chromosome which display the symptoms of X-linked hypophosphatemia: bone changes resembling rickets, dwarfism (smaller body mass and shorter tail), and decreased renal reabsorption of phosphate. Further studies have shown that *Hyp* mice have the same reduced renal tubular transport of phosphate (2, 3) and osteomalacic bone disease (4) as those seen in human patients (5, 6).

Most investigations involving *Hyp* mice have involved the skeletal, renal, endocrine, or gastrointestinal systems. Investigations into other physiological systems in *Hyp* mice might prove useful in elucidating the mechanisms underlying the disease. We have previously reported increased food consumption per gram body weight in *Hyp* mice compared to normal mice (7, 8). The present study was undertaken to examine whether there is some abnormality in the metabolism of *Hyp* mice which is responsible for increased food intake. We measured plasma free T_4 levels, oxygen consumption, and distribution of cardiac output and determined the effect of a low phosphate diet, age, and sex on oxygen consumption in *Hyp* and normal C57BL/6J mice.

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Materials and Methods

Animals

Normal and hypophosphatemic C57BL/6J mice were bred in our laboratory as previously described (7). The mice were maintained at 24 C on a 14-h day, 10-h night cycle and were fed Wayne Lab Blox (Allied Mills, Inc., Chicago, IL) after weaning and tap water. Littermates were used in all experiments. Heterozygous-hypophosphatemic females (*Hyp*/+) and hemizygous-hypophosphatemic males (*Hyp*-/Y) show the same low renal tubule reabsorption of phosphate (9), hypophosphatemia (2), increased urinary CAMP (10), changes in vitamin D metabolism (11), and duodenal malabsorption of ^{45}Ca in young animals (12). Because of the similarities in the disease in both sexes and because of the limited availability of animals, both sexes were used.

Distribution of cardiac output

We used a method similar to a previously reported study in which distribution of cardiac output was measured in mice (13).

Twelve-week-old *Hyp* and normal mice were anesthetized with ether. The left ventricle of the heart was punctured with a 0.5-in. 26-gauge needle attached to a polyethylene cannula (PE20) filled with 0.9% NaCl (with a dead space of 0.1 ml). The mice were infused with 15-μm diameter microspheres labeled with Cerium-141 (New England Nuclear, Boston, MA). Approximately 40,000 spheres were infused in 0.1 ml 0.9% NaCl with 0.01% Tween-80. The spheres were sonicated for 60 min and vortexed for 5 min before injection to minimize clumping. The mice were infused at a rate of 0.2 ml/min for 1 min. The first 0.1 ml injected solution was saline from the tubing dead space, and the second 0.1 ml contained the microspheres. After a 5-min period, the animals were killed with ether, and the mice were dissected, and the tissues were counted using a Beckman 7000 γ-scintillation counter (Beckman, Palo Alto, CA).

Measurement of oxygen consumption

Oxygen consumption was measured using a closed circuit apparatus. Adult (10 weeks) male and young (4–5 weeks) male and female mice were placed in plexiglass cylindrical chambers (20 cm long × 9 cm in diameter) surrounded by water which was regulated at 28 C. The chambers were connected via short tubes to bells containing oxygen. Expired carbon dioxide was absorbed with soda lime (Fisher Scientific Co., Fairlawn, NJ). The bells floated in the water above the chambers and gradually sank into the water as oxygen was consumed by the mice. The movement of the bells was calibrated so that the volume of gas in the bell could be continually monitored. Mice were observed continually, and bell volumes were recorded when the mice were sleeping to determine resting oxygen consumption. The mice were placed in the chamber 2 h/day for 4 days before the experimental day to adapt them to the chamber.

T_4 levels

Free T_4 was measured using an Amersham RIA kit (IM.2051, Amersham, Arlington Heights, IL). Plasma was collected from 14-week-old normal and heterozygous *Hyp* female mice from the intraorbital sinus via heparinized capillary tubes.

Low phosphate diet

The low phosphate diet used was ICN low phosphorus diet (no. 902206, ICN Pharmaceuticals, Inc., Cleveland, OH) which contains 0.02% phosphate and 0.45% calcium. To produce a normal phosphate diet, 39.8 g $Na_2HPO_4 \cdot 7H_2O$ and 6.2 g KH_2PO_4 were added to 954 g of the low phosphate diet to give a diet containing 0.6% phosphate.

Normal and *Hyp* male and female adult (9–10 weeks) mice (mostly littermates) were subdivided into four groups: normal mice fed a normal diet, normal mice fed a low phosphate diet, *Hyp* mice fed a normal diet, and *Hyp* mice fed a low phosphate diet. The mice were kept on these diets for 2 days, and then their oxygen consumption was measured. This method has been used previously to produce low plasma phosphate levels (11).

The mice were anesthetized with ether, and blood samples were drawn from the intraorbital sinus into heparinized capillary tubes. Plasma inorganic phosphate was measured by a colorimetric procedure (14).

Measurement of surface area

Adult (13–15 weeks) male mice were killed with ether, weighed, and skinned; the pelt outline was traced on graph paper; and the surface area was measured graphically.

Statistics

Data were analyzed using Student's t test or factorial analysis of variance. If after analysis of variance, identification of statistical differences between the means of groups was desired, Duncan's multiple range test or nonparametric multiple comparisons test (15) was performed depending on the outcome of Cochran's test for homogeneity of variance. Data in the text and tables are presented in the form of means ± SE.

Results

Hyp mice had significantly greater oxygen consumption than normal mice (Table 1). This difference was present in young and adult mice and in both male and female *Hyp* mice. There was no difference in metabolic rate between male and female mice of either genotype.

There were significant differences in body weight between *Hyp* and normal mice at both 4–5 and 10 weeks of age. Normal adult mice weighed 25.3 ± 0.4 g, and *Hyp* mice weighed 19.9 ± 0.6 g ($P < 0.0005$, by Student's t test). Normal young mice weighed 13.9 ± 0.4 and *Hyp* mice weighed 11.8 ± 0.4 g ($P < 0.01$, by Student's t test).

To test whether there might be increased heat loss due to a greater surface area to body mass ratio in *Hyp* mice, the surface area of *Hyp* and normal mice was measured. Because of the shorter tails and limbs of the *Hyp* mice, the surface area to mass ratios were not different [2.36 ± 0.07 cm^2/g in normal mice (n = 4) *vs.* 2.39 ± 0.17 cm^2/g in *Hyp* mice (n = 4)]. Metabolic rate per unit weight generally declines with increasing weight according to the equation $M = K \cdot W^{-0.27}$, where M is the metabolic rate, W is the body weight, and K is a proportionality constant (13). Therefore, to determine metabolic rate

TABLE 1. Oxygen consumption in *Hyp* and normal mice

Age (weeks)	Normal		*Hyp*	
	Male	Female	Male	Female
10[a,b,c]	38.1 ± 1.2 (7)		49.5 ± 1.6 (6)	
4–5[b,c,d]	56.7 ± 3.2 (3)	56.3 ± 0.8 (4)	63.0 ± 3.1 (4)	61.3 ± 2.4 (4)

Oxygen consumption is expressed as microliters of O_2 per min/g BW. The data were analyzed by factorial analysis of variance. The number of animals is in parentheses.

[a] Age effect, $P < 0.00001$.

[b] Genotype effect, $P < 0.00004$.

[c] All two-way interactions were nonsignificant.

[d] Sex effect, $P > 0.67$.

which is independent of body weight, one can express metabolic rate in units of oxygen consumed per time/$wt^{.73}$ (13, 14). When the oxygen consumptions from adult *Hyp* and normal mice were compared using these units, there was still a significant difference (111.1 ± 3.5 μl O_2/$min \cdot g^{.73}$ in *Hyp* mice *vs.* 91.2 ± 3.4 μl $O_2/min \cdot g^{.73}$ in normal mice; $P < 0.01$, by Student's t test). The difference in oxygen consumption between *Hyp* and normal young animals was not as great as in the adults and using this conservative estimate of oxygen consumption, the difference between oxygen consumption in *Hyp* and normal young mice was not significant (121.0 ± 4.0 μl O_2/$min/g^{.73}$ in *Hyp* mice *vs.* 114.7 ± 2.2 μl $O_2/min/g^{.73}$ in normal mice, $P > 0.10$, Student's t test).

To test whether the hypophosphatemia *per se* caused the altered metabolic rate, a low phosphate diet was fed to normal and *Hyp* mice. Placing the mice on the low phosphate diet for 2 days reduced the blood phosphate level in normal mice to levels comparable to those in *Hyp* mice receiving the control diet (Table 2). A reduction in plasma phosphate did not produce a difference in oxygen consumption in either *Hyp* or normal mice from littermates fed the control diet consisting of normal phosphate levels (Table 2). The sex of the animal did not effect either the oxygen consumption ($P > 0.07$, factorial analysis of variance) or the plasma phosphate levels ($P > 0.98$, factorial analysis of variance).

To test whether a difference in thyroid hormone levels was responsible for the increased metabolic rates, plasma free T_4 levels were measured. There was no difference between *Hyp* and normal mice (10.1 ± 0.9 pmol/liter in normal mice *vs.* 10.1 ± 0.7 pmol/liter in *Hyp* mice.)

To determine whether the altered metabolic rate was associated with altered distribution of cardiac output, 15-μm diameter, Ce-141-labeled microspheres were infused into the left ventricles of *Hyp* and normal mice. The results are shown in Table 3. Distribution of cardiac output to the kidneys, heart, brain, spleen, and gonads was similar in *Hyp* and normal mice. However, there were significant differences in blood flow to liver and gastrointestinal tract and to skin, muscle, and bone.

TABLE 2. Effect of low phosphate (P) diet on oxygen consumption in normal and *Hyp* mice

	Normal mice		*Hyp* mice	
	Control diet (n = 7)	Low P diet (n = 8)	Control diet (n = 11)	Low P diet (n = 9)
O_2 consumption ($\mu l/min \cdot g$)[a,b,c]	49.8 ± 4.3	51.5 ± 2.0	59.7 ± 2.0	58.4 ± 1.7
Plasma P (mM)[c,d,e]	2.18 ± 0.05	1.55 ± 0.19[f]	1.28 ± 0.05[f]	0.76 ± 0.12

[a] Genotype effect, $P < 0.006$ (factorial analysis of variance).
[b] Diet effect, $P > 0.98$ (factorial analysis of variance).
[c] All two-way interactions were nonsignificant.
[d] Genotype effect, $P < 0.001$ (factorial analysis of variance).
[e] Diet effect, $P < 0.001$ (factorial analysis of variance).
[f] Plasma P levels of normal mice on a low P diet were not significantly different from those in *Hyp* mice on a control diet ($P > 0.5$, nonparametric multiple comparisons).

Discussion

We have previously reported that *Hyp* mice have increased food consumption compared to normal mice (7, 8). The present study has demonstrated that this can be explained by an increased metabolic rate in the *Hyp* mice. It is unclear at this point what is causing the increased metabolic rate.

Acute changes in plasma phosphate did not alter the metabolic rate of *Hyp* or normal mice, suggesting that the increased metabolic rate of the *Hyp* mice is not due to their hypophosphatemia. Chronic abnormalities in plasma phosphate and other associated variables such as vitamin D and PTH are seen in patients with uremia and other conditions such as diabetes, burns, and respiratory alkalosis (16). These conditions may produce decreased glycolytic activity and impaired anerobic energy metabolism (17) which may be due to among other things, altered ATP content (18) or a change in 2,3-DPG levels (19). When rats are placed on rachitic diets for several weeks, there is either no change in metabolic rate as the rats develop rickets (20) or the rats show a de-

TABLE 3. Distribution of cardiac output in normal and *Hyp* mice

Tissue	Normal (n = 5)	*Hyp* (n = 5)
Liver	0.74 ± 0.21	1.26 ± 0.11[a]
Spleen	0.32 ± 0.08	0.17 ± 0.03
Gastrointestinal tract	24.50 ± 0.80	17.83 ± 1.04[b]
Kidneys	12.76 ± 1.07	10.14 ± 1.24
Gonads	0.41 ± 0.08	0.49 ± 0.10
Heart	9.67 ± 1.84	11.31 ± 1.87
Brain	6.16 ± 1.44	6.82 ± 0.90
Tail	0.33 ± 0.06	0.47 ± 0.08
Skin[c,d]	0.52 ± 0.07	0.87 ± 0.04[b]
Muscle[c,e]	1.22 ± 0.09	1.76 ± 0.11[f]
Bone[c,g]	2.28 ± 0.24	3.56 ± 0.21[h]
Remaining carcass	37.44 ± 4.39	43.76 ± 4.05

Values represent the percentage of cardiac output, except as noted. The data were analyzed by Student's t test.
[a] $P < 0.05$.
[b] $P < 0.001$.
[c] Values represent the percentage of cardiac output per g, since whole organs were not obtained.
[d] Skin from hind limbs to pectoral girdle.
[e] Hind limb and ventral abdominal muscles.
[f] $P < 0.025$.
[g] Bones from hind limb and calvaria.
[h] $P < 0.0025$.

pressed metabolic rate (21). There does not seem to be support, therefore, for the involvement of chronic hypophosphatemia in increased metabolic rate.

The increased metabolic rate also is apparently not due to abnormal T_4 levels, at least in female mice. Thyroid involvement is still possible since T_4 levels were not determined in male mice, since other thyroid hormones have not been measured, and since such factors as tissue responsive and turnover rate have not been measured.

The increased metabolic rate is also not due to the differences in body weight between *Hyp* and normal mice since there was a difference in metabolic rate in adult animals even when metabolic rate was calculated in a weight-independent manner (22, 23). Also, direct measurement of surface area to weight ratios (which could affect rates of heat loss) demonstrated that the ratios were not different.

Hyp mice may differ from normal mice in heat loss due to other factors, however. Effective surface area due to postural adjustments may differ, the ratio of conductive to nonconductive surface areas may be different, and the amount of insulation (fat, fur) may differ. Also, physiological control of heat loss via vasomotor tone may differ.

The results of the distribution of cardiac output experiment suggest an increased blood flow to thermogenic organs (liver and muscle) and increased blood flow to sites of heat loss (skin), with a consequent decrease in blood flow to the gastrointestinal tract. This suggests several possible explanations for the increased metabolic rate. It is possible that there is an alteration in the central thermoregulatory control centers which may be causing increased heat loss and/or increased metabolic rate. There might also be an alteration in the cardiovascular system, either centrally or peripherally, with a decreased ability to vasoconstrict the tail and skin vasculature. This would result in increased heat loss and a compensatory increased metabolic rate. A third possibility is that there might be a primary metabolic defect or a metabolic defect due to abnormal hormonal activities. Further investigations are underway to answer these questions.

This study raises questions regarding the role of the increased metabolic rate and the altered distribution of cardiac output in the development of the symptoms of X-linked hypophosphatemia. Does a decreased blood flow to the gastrointestinal tract play a role in the decreased calcium absorption that has been reported in young *Hyp* mice (7)? Does an increased blood flow to bone affect its growth rate or the development of osteomalacia? Does the increased metabolic rate itself have a growth-stunting effect? These questions remained unanswered.

Also of interest is the possibility that understanding the underlying defect responsible for the increased metabolic rate and/or the altered distribution of cardiac output may help in understanding the mechanisms involved in X-linked hypophosphatemia. In the past, research on X-linked hypophosphatemia was largely focused on renal, endocrine, and skeletal aspects of the disease. Recently, research has expanded into other areas, such as the involvement of the gastrointestinal tract (7, 24, 25). Study of other systems, such as the cardiovascular and thermoregulatory systems, may help elucidate the defects responsible for this disease.

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