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Modulation of insulin-like growth factor-I: A specific role for vitamin B_1 (thiamine)

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The aim of the present study was to determine if the impairment in growth and weight gain observed with thiamine deficiency is associated with alterations in plasma and tissue levels of insulin-like growth factor-I (IGF-I). Male rats were fed a thiamine-deficient (TD) or pair-fed a nutritionally complete (C) purified diet for either 2 or 6 weeks. There was no difference in weight gain between TD and C animals at 2 weeks. Thereafter, weight gain for the two groups diverged and was 35% lower in the TD than in C rats after 6 weeks. The blood total thiamine concentration was reduced by 76% and more than 90% at 2 and 6 weeks, respectively. Although the percent of total thiamine in the pyrophosphate form was not changed in the TD group (35 to 38%), the thiamine monophosphate (TMP) form was not detectable by 6 weeks and the percent in thiamine measured as its mononitrate (TMN) form had increased from 25% in control rats to 62% after 6 weeks on the TD diet. Plasma growth hormone levels were similarly reduced after 2 and 6 weeks (70 to 85%) on the TD diet. In TD rats, plasma IGF-I was decreased 28% (2 weeks) and 40% (6 weeks). Tissue IGF-I content of TD rats decreased at 2 and 6 wk in kidney (62% and 60%), liver (30% and 54%), muscle (21% and 52%), brain (41% and 56%), and pituitary (40% and 42%). Plasma levels of IGF binding proteins (BP-1/2, BP-3, and a small molecular weight BP [28 kDa]) of TD rats were decreased approximately 65% at 2 weeks and remained reduced at 6 weeks. The depression of the IGF system in TD at 2 weeks was not associated with changes in either plasma insulin or corticosterone concentrations; at 6 weeks, however, insulin was reduced by 30% and corticosterone increased by 90%. These results suggest a role for thiamine in the modulation of the IGF system, which is independent of changes in caloric intake and changes in the plasma concentration of insulin or corticosterone. (J. Nutr. Biochem. 7:207–213, 1996.)

Keywords: thiamine deficiency; IGF-I; IGF binding proteins; growth; rats; growth hormone; insulin; corticosterone

Introduction

Thiamine (vitamin B_1), a water-soluble vitamin that is converted to thiamine pyrophosphate in the presence of ATP, functions as a cofactor for several enzymes involved in intermediary metabolism. Among these, pyruvate dehydrogenase and α-ketoglutarate dehydrogenase are key enzymes directly involved in conversion of glucose to energy, CO_2, and H_2O.[1] Thus, this vitamin plays a crucial role regulating energy metabolism. Reports in the literature indicate that animal models of thiamine deficiency are associated with impaired weight gain primarily due to decreased appetite in the deficient animals.[2,3] Previous studies from our laboratory[4] demonstrated that even under conditions of pair-feeding, the rate of weight gain in the thiamine-deficient animals was significantly impaired compared with animals fed a nutritionally complete diet that was isocaloric and isonitrogenous. This suggests that in thiamine deficiency the impairment in weight gain may be regulated by factors other than caloric intake, most likely related to alterations in anabolic hormones.

It is well accepted that nutrition is one of the main modulators of circulating insulin-like growth factor (IGF)-I.[5,6]

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Received August 24, 1995; accepted December 6, 1995.

Nutritional Biochemistry 7:207–213, 1996

655 Avenue of the Americas, New York, NY 10010

0955-2863/96/$15.00
PII S0955-2863(96)00011-3

Both dietary energy and protein are critical in the regulation of serum IGF-I concentrations. Numerous studies have demonstrated that plasma IGF-I concentrations are reduced by fasting and are restored with refeeding. Whereas the lowest protein intake (0.2 g/kg/day) is able to increase IGF-I in the presence of adequate energy, there is a threshold energy requirement below which optimal protein intake (1 g/kg/day) fails to raise IGF-I after fasting.[7] Furthermore, when energy intake is severely reduced, the carbohydrate content of the diet is a major determinant of responsiveness of IGF-I to the stimulatory effects of growth hormone (GH).

The mechanisms responsible for the effects of nutrition on GH secretion are poorly understood and the effects of prolonged changes in dietary intake on GH release are not known.[8,9] Whereas caloric restriction increases GH release in most mammals, including humans, in the rat, caloric restriction results in a decrease in GH release. Furthermore, whereas in sheep and steers only the amplitude of GH pulses increases, both the number and amplitude of GH pulses increases in dogs. Decreases in circulating levels of IGF-I resulting from food deprivation are associated with significant elevations in plasma GH. It has been hypothesized that rapid changes in plasma IGF binding protein (BP) concentrations may mediate the effect of nutrition on GH secretion by regulating the amount of unbound or bioavailable IGF-I in plasma. In support of this hypothesis, IGFBP-1 concentrations are increased by fasting and decrease rapidly after refeeding. Currently, there is no information available on the specific nutrient requirements for intact GH/IGF-I axis.

The purpose of the present study was to determine if the impaired growth observed during thiamine deficiency is associated with alterations in the circulating and tissue levels of IGF-I and IGFBPs. In addition, plasma concentrations of known modulators of the IGF system (i.e., GH, insulin and corticosterone) were determined to assess their potential role in control of IGF-I and the IGFBPs during specific nutrient (vitamin) deprivation.

Methods and materials

Animal preparation

Male Sprague-Dawley rats (175–200 g, Charles River, Wilmington, MA, USA) were housed in a controlled environment, exposed to a 12:12 hr light-dark cycle and fed standard rat chow (Purina) for 1 week before being randomly placed on the test diet. Animals were then fed a thiamine-deficient diet (5833 M; Purina Mills, St. Louis, MO, USA) that contained RP Vitamin Mix without thiamine (60% carbohydrate, 21% protein, 10% fat). The rats on the control diet were pair-fed a diet identical in composition except for the content of thiamine.[4] All rats were housed individually in metabolic cages for the duration of the experimental protocol. At the end of either 2 or 6 weeks, rats (n = 6–7 per group) were anesthetized with ether, and blood samples withdrawn by cardiac puncture, and selected tissues excised, freeze clamped and stored at −80°C until analyzed. Thiamine mononitrate (TMN) and monophosphate (TMP) concentrations were determined in plasma and the active form of the vitamin (i.e., coenzyme), thiamine pyrophosphate (TPP), was determined in red blood cells (RBC). Because values for control rats did not vary over the duration of the experiment, control values at 2 (n = 6) and 6 (n = 7) weeks were combined. All experiments were approved by the Animal Care and Use Committee of The State University of New York at Stony Brook. These studies adhered to the National Institutes of Health guidelines for the use of experimental animals.

Analysis of plasma, RBC, and tissue thiamine

Analysis of erythrocyte TPP and TMP was done by high performance liquid chromatography (HPLC, Beckman Instruments Model 126 System Gold), using a modified version of the method described by Warnock.[10] TMN (Sigma Chemical Co., St. Louis, MO, USA) was used as an internal standard. Blood was drawn into a tube containing EDTA as an anticoagulant, centrifuged for 5 min at 2,500 rpm, and the plasma was removed and stored at −80°C for further analysis. RBCs were washed three times with cold 0.9% w/v saline and washed cells stored at −20°C until preparation for analysis.

Preparation of samples for analysis

To 500 μL of washed red cells, 50 μL of internal standard was added and the sample vortexed. To this mixture, 410 μL of 10% w/v TCA was added, samples vortexed, centrifuged at 25,000 rpm for 15 min, and the supernatant removed. The supernatant was washed three times with twice the volume of water-saturated ether to remove TCA (i.e., to 500 μL supernatant add 1 ml of ether). To this, 40 μL of 1% w/v potassium ferricyanide solution was added and then filtered through a 0.45 μm Gelman LC 13 Acrodisc.

IGF-I determination

Plasma was extracted using a modified acid-ethanol procedure, and tissues were extracted using acid homogenization and Sep-pak (C_{18}) extraction, as previously described.[11] IGF-I in plasma and tissues was determined by radioimmunoassay (RIA). Recombinant human [Thr^{59}]IGF-I (gift of Upstate Biotechnology Incorporated, Lake Placid, New York, USA) was used for iodination and standards, as previously described.[11] The ED_{50} for this assay is 0.03–0.08 ng/tube; inter- and intra-assay coefficients of variation are 10% and 7%, respectively.

Ligand blotting

Binding proteins in plasma and tissues were determined by Western ligand blot analysis, as described by Hossenlopp et al.[12] and slightly modified by our laboratory.[13] Protein content in the supernatant was assayed to ensure that each sample had an equal concentration of protein. Samples were subjected to SDS-PAGE without reduction of disulfide bonds. The electrophoresed proteins were transferred overnight onto nitrocellulose in Tris-methanol-glycine buffer. Nitrocellulose sheets were washed and then incubated overnight with radiolabeled IGF-I with and without excess unlabeled IGF-I. The nitrocellulose sheets were washed extensively in Tween 20, dried, and autoradiographed with X-ray film (Kodak X-Omat AR, Eastman Kodak Co., Rochester, NY, USA) and intensifying screens (DuPont, Wilmington, DE, USA) at −70°C for 2 to 4 days. Autoradiographs were quantified using a laser densitometer.[13]

Affinity labeling and immunoprecipitation for binding proteins

On the ligand blots, IGFBP-1 and -2 appear as a band at 30,000 to 32,000 M_r and cannot be easily resolved. Therefore, specific antisera to human IGFBP-1 and bovine IGFBP-2, which cross-react

with rat BPs,[11,12] was used to perform immunoprecipitation (generously provided by Dr. D. Clemmons, University of North Carolina, Chapel Hill, NC, USA). IGFBP-3, which appears as a complex in the range of 47,000 to 53,000 M_r, and a 28,000 M_r band, were not further characterized. The tissue and plasma samples were prepared as previously described. An aliquot of homogenate or diluted plasma was used and incubated with radiolabeled IGF-I, with or without 1 μg unlabeled IGF-I. IGFBP complexes were cross-linked with disuccinimidyl suberate and terminated by addition of Tris-HCl. For immunoprecipitation, either antiserum to IGFBP-1 or -2 was added for overnight incubation. Antibody-bound complexes were precipitated with goat anti-rabbit IgG followed by the addition of polyethylene glycol 6,000. Samples were centrifuged and the supernatant removed. The pellets were rinsed twice with phosphate buffer, solubilized in sample buffer, boiled, and then loaded on 10% SDS-PAGE. The gel was washed, dried, and exposed to X-ray film at −70°C for 2 to 4 days. All ligand blots were quantified by two-dimensional laser scanning densitometry (three to five scans per blot; coefficient of variation <5%; Hoefer; San Francisco, CA, USA). Affinity labeling and immunoprecipitation for IGFBP-1 and -2 was determined for tissues and plasma from three different LPS- and saline-injected rats.

Plasma hormone concentrations

Blood samples for determination of plasma hormone levels were collected in chilled syringes containing aprotinin (500 KIU/ml) and the plasma stored at −80°C until assayed. Plasma immunoreactive insulin was measured by RIA using a double antibody method (DPC, Los Angeles, CA, USA). The interassay coefficient of variation (CV) ranged from 6.8 to 13.4%. Plasma corticosterone was assayed using a double antibody kit method from DPC, with interassay CV from 6.1 to 6.3%. Plasma GH concentrations were determined by RIA using a rat GH [^{125}I] assay system (Amersham; Arlington Heights, IL, USA).

Statistics

Experimental values are presented as means ± SEM. Values for plasma hormone concentrations and total thiamine content of pair-fed controls did not differ between those animals sacrificed at 2 and 6 weeks. Therefore, values from all time-matched pair-fed controls were combined and averaged. Data were analyzed either by analysis of variance followed by Student-Newman-Keuls test to determine treatment effects or Student's *t*-test. Statistical significance was set at $P < 0.05$.

Results

Body weight and food consumption

During the first 2 weeks on the diet, there was no significant difference in the rate of weight gain between the deficient and the control animals (*Figure 1*). No difference in body weight gain was detected after 2 weeks on the diet (deficient; 83 ± 8 g versus control; 97 ± 9 g). Thereafter, weight gain for the two groups diverged, and after 6 weeks on the diet the weight gain of the deficient animals was approximately 35% lower than that of the control rats. As a result, the body weight at the end of the 6-week period on the diet was 20% lower in the TD rats than that of pair-fed control animals. Food consumption averaged 20 ± 2 g/d for TD rats and 21 ± 3 g/d for the pair-fed control rats. These averages did not vary significantly over the course of the study for either group.

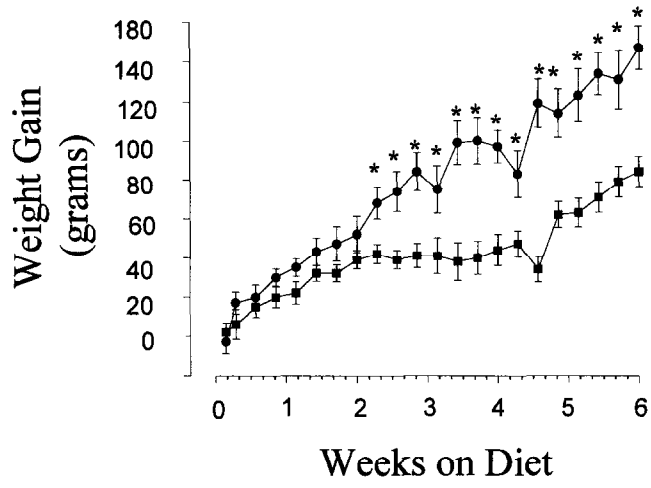

Figure 1 Cumulative weight gain over time on the diet. Values are means ± SEM. Circles (●) correspond to values of animals on the pair-fed control diet and squares (■) represent values of rats on the thiamine-deficient diet. *P <0.05 compared to thiamine-deficient animals (n = 6 to 11 per group).

Blood thiamine levels

Total blood thiamine in the control animals averaged 2300 ± 208nM and was distributed as thiamine pyrophosphate (TPP; 35%), thiamine mononitrate (TMN; 25%), and thiamine monophosphate (TMP; 39%) (*Figure 2*). After 2 weeks on the diet, the total blood thiamine concentration was decreased by 76%, compared with control values. The percentage in the active form of the vitamin (TPP) remained constant. However, there was an increase in the percentage of TMN (43%) and a decrease in that of TMP (20%). After 6 weeks on the deficient diet, total thiamine levels in blood were 10% of control values. At this time, TMP was not detectable in blood samples from thiamine-deficient rats. The percentage of total thiamine in the TPP form remained constant (38%), whereas that in the form of TMN increased to 62%.

Hormone concentrations

Two weeks on the deficient diet did not significantly alter the circulating levels of either insulin or corticosterone, compared to pair-fed control values. Consumption of the thiamine-deficient diet for 6 weeks resulted in significant reduction of insulin concentrations (30%) and higher corticosterone levels (95%), compared with control values. Plasma levels of GH were decreased 70% after 2 weeks on the thiamine-deficient diet and were further reduced after 6 weeks on the diet.

Plasma and tissue IGF-I content

Circulating levels of IGF-I were reduced 30% at 2 weeks and 40% after 6 weeks on the thiamine-deficient diet (*Figure 3*). Liver content of IGF-I was the highest among the tissues analyzed and averaged 240 ± 13 ng/g in the control animals. Thiamine deficiency decreased the hepatic IGF-I content to an average of 168 ± 14 ng/g at 2 weeks. Liver was the only tissue that showed a further decrease after 6 weeks

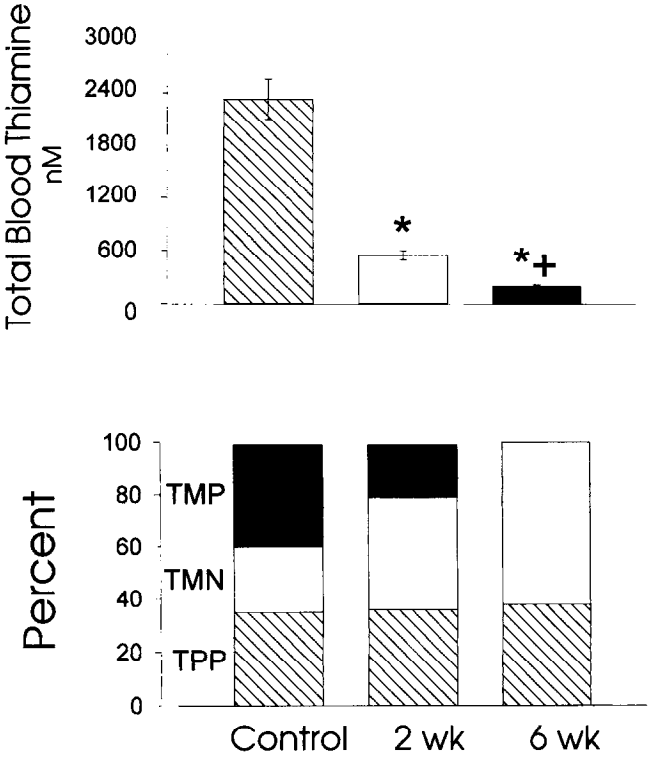

Figure 2 Top panel (A). Total blood thiamine concentrations in pair-fed control (shaded bars) and thiamine deficient rats expressed as nM after 2 weeks (open bars) and 6 weeks (filled bars) on the diet. Values are means ± SEM (n = 6 to 11 per group). *P < 0.05 compared with control values. Bottom panel (B). Distribution of total circulating thiamine in thiamine pyrophosphate (TPP; shaded bar), thiamine mononitrate (TMN; open bar), and thiamine monophosphate (TMP; filled bar) in pair-fed control and thiamine-deficient animals after 2 and 6 weeks on the diet. Values are expressed as percent of total thiamine. n = 6 to 11 per group.

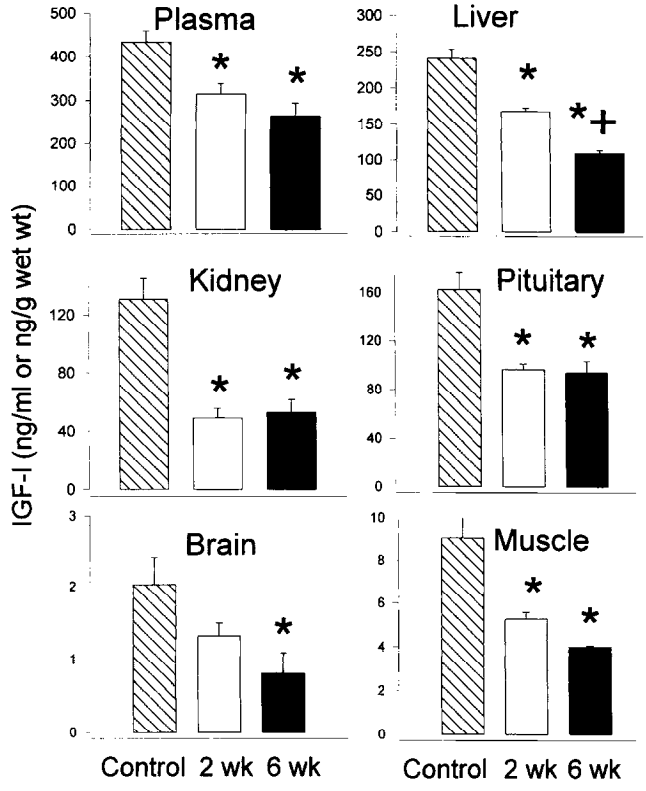

Figure 3 Plasma, liver, kidney, pituitary, brain and muscle concentrations of IGF-I in control (shaded bars), 2 weeks (open bars), and 6 weeks (filled bars) thiamine-deficient rats expressed as either ng/ml (plasma) or ng/g wet weight (tissues). Values are means ± SEM. n = 6 to 11 per group, *P < 0.05 compared to controls.

on the thiamine-deficient diet, averaging 109 ± 5 ng/g (P < 0.05) at this time. IGF-I content in the kidney averaged 131 ± 14 ng/g in the control rats and dropped 62% after 2 weeks, and was similarly depressed after 6 weeks on the diet. Pituitary levels of IGF-I were 162 ± 14 ng/g in the control rats and decreased significantly to 97 ± 4 ng/g after 2 weeks on the thiamine deficient diet. These levels failed to show any further decrease after 6 weeks on the diet. Brain content of IGF-I decreased approximately 40% after 2 weeks and showed a further, though not statistically significant, decrease (56%) after 6 weeks. Thiamine deficiency decreased muscle IGF-I after 2 weeks, and muscle IGF-I content remained reduced 50% after 6 weeks, compared with control values (P < 0.05).

TABLE 1 Hormonal alterations in thiamine deficiency

	Pair-fed Control	Deficient 2 weeks	Deficient 6 weeks
Insulin (pM)	228 ± 18	216 ± 12	150 ± 18*†
Growth hormone (μg/L)	25.2 ± 2.7	7.2 ± 1.5*	3.9 ± 0.3*†
Corticosterone (nM)	474 ± 121	514 ± 66	855 ± 113*†

Hormonal alterations in response to thiamine deficiency after 2 and 6 weeks on the diet, presented as mean ± SEM.
*P < 0.05 compared to pair-fed control values.
†P < 0.05 compared to 2 week deficient values.
Control values represent the average of values of pair-fed controls at 2 and 6 weeks. n = 6 to 11 per group.

IGF binding proteins

Three major IGFBPs were identified by ligand blotting of plasma obtained from both control and thiamine-deficient rats (*Figure 4*). These bands had a molecular weight (M_r) of 47,000–53,000 kDa (and most likely represents IGFBP-3), 32,000 (IGFBP-1 and/or -2) and 28,000 kDa. Because the M_r of IGFBP-1 and -2 are similar, these BPs are not easily separated by ligand blotting. Thus, the 32,000 M_r band on the ligand blot is referred to as IGFBP-1/2. It is clear from the autoradiograph that all the major IGFBPs identified by ligand blotting were decreased in rats after 6 weeks on the thiamine-deficient diet. Similar changes in IGFBPs were seen after 2 weeks on the thiamine-deficient diet (data not shown).

To obtain a quantitative assessment of the magnitude of the decrease in circulating IGFBP levels, densitometry was performed on the ligand blots (*Figure 5*). Plasma IGFBP-1/2 showed an initial dramatic decrease to 33% of control

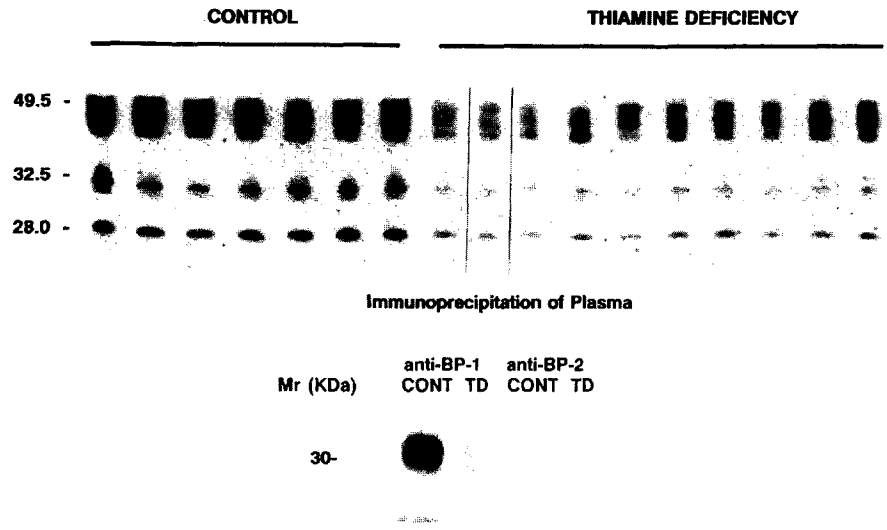

Figure 4 Panel A (top). Ligand blot of IGF binding proteins in plasma from pair-fed controls and 6-week thiamine-deficient rats. For all ligand blots, M_r standards were lysozyme (14,300), carbonic anhydrase (30,000), ovalbumin (46,000), bovine serum albumin (69,000) and phosphorylase b (92,500) (not shown). Panel B (bottom). Affinity cross-linking and immunoprecipitation with antiserum to IGFBP-1 and -2 were performed on plasma from control and thiamine-deficient rats. Nonspecific binding was determined by adding an excess (1 μg) of unlabeled IGF-I (data not shown). Affinity cross-linking and immunoprecipitation was performed on plasma obtained from 3 different animals after 6 wk of thiamine deficiency. All samples demonstrated a decrease in IGFBP-1. This panel illustrates a representative autoradiograph of plasma from one control and one thiamine-deficient animal.

values after 2 weeks on the diet and was similarly reduced after 6 weeks on the diet. Subsequent immunoprecipitation indicated that the decrease in IGFBP-1/2 in plasma was predominantly due to a decrease in IGFBP-1 (*Figure 4*, bottom). Plasma IGFBP-3 levels dropped to 38% at 2 weeks and 58% of control values at 6 weeks. IGFBP-28K levels decreased to 35% of control values after 2 weeks and remained similarly reduced (39%) after 6 weeks on the diet.

Immunoprecipitation of liver demonstrated that thiamine deficiency resulted in a preferential decrease in IGFBP-1 (*Figure 6*). In contrast to this, IGFBP-1 in muscle appeared to be comparable in the control and thiamine-deficient animals (*Figure 6*).

Discussion

The findings of the present study suggest a significant role of IGF-I and its binding proteins in modulating the growth retardation associated with a single vitamin deficiency, namely that of thiamine. Interestingly, in this model, biochemical evidence of thiamine deficiency was noted after 1 week when circulating thiamine levels have been shown to be decreased.[4] Progression of the thiamine-deficient state was associated with marked reductions in total blood thiamine (76% by 2 weeks and 90% by 6 weeks), but with preservation of the percentage of the active component (≈ 40%), TPP, and a redistribution of the ratio of TMN to TMP.

Previous reports have suggested that the impaired growth rates for rats on a thiamine-deficient diet results from decreased appetite and the accompanying decrease in food intake.[2] The results of the present study, although they do not refute this hypothesis, suggest that an additional mechanism is operational during thiamine deficiency that is independent of energy or nutrient intake, and appears to involve the GH/IGF-I axis.

Although it is well accepted that both dietary energy and protein are critical in the regulation of serum IGF-I concentrations,[5,6,7] the impact of a specific nutrient, specifically that of vitamins, on the regulation of the GH/IGF-I axis has not been previously examined. During thiamine deficiency the alterations in circulating and tissue levels of IGF-I may be attributed to the low GH levels, the suppressed GH levels are unexplained. Furthermore, the mechanisms responsible for the effects of nutrition on GH secretion are poorly understood and the effects of prolonged changes in dietary intake on GH release are not known.[8,9]

Several conclusions can be reached from the present study regarding the inter-relationship between thiamine deficiency and tissue and plasma concentrations of IGF-I. The drop in plasma and tissue IGF-I levels occurred after 2 weeks; and involved all tissues examined. Marked early reductions were noted in the kidney (≈ 60% at 2 weeks) and in the pituitary, and these reduced levels were sustained by 6 weeks on the diet. Significant reductions in tissue IGF-I also occurred in the liver, brain, and skeletal muscle. The mechanism for such alterations could, in many ways, be hormonally-mediated and/or be the result of modulation in the relationship between IGF-I and its binding proteins. It is well established that the large majority of circulating IGF-I in humans and rats is bound to IGFBP-3, which has a M_r of 47,000-53,000. However, at least five other smaller IGFBPs also appear to be present in body fluids.[14] These IGFBPs are produced in a variety of tissues and have been shown either to enhance or to inhibit selected effects of IGF-I.[15]

Several hormones have been shown to modulate IGF-I levels in blood and tissues. Of these, GH appears to play an important role in modulating the circulating levels of

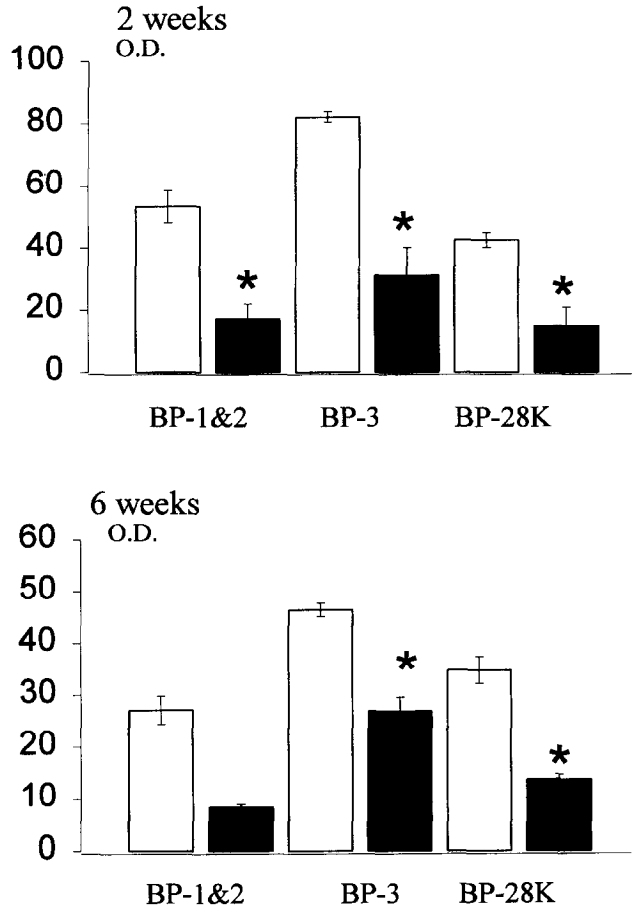

Figure 5 Plasma levels of IGF binding proteins in control and 2-week (top panel) and 6-week (bottom panel) thiamine-deficient rats, expressed as optical density (O.D.). Values are means ± SEM. n = 6 to 11 per group, $^*P < 0.05$ compared with controls.

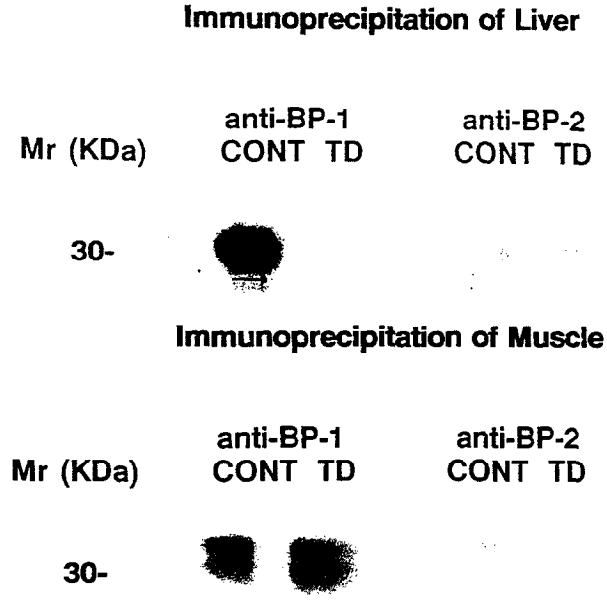

Figure 6 Panel A: Immunoprecipitation of IGF binding proteins in liver (A) and muscle (B) from control and thiamine-deficient animals. Affinity cross-linking and immunoprecipitation were performed on liver and gastrocnemius muscle obtained from three different control and thiamine-deficient animals. All three livers demonstrated a decrease in IGFBP-1 and no change in IGFBP-2. In skeletal muscle, IGFBP-1 and -2 were comparable in the control and in the thiamine-deficient animals. This figure illustrates a representative autoradiogram of liver and muscle.

IGFBP-3 and of the circulating and tissue levels of IGF-I during thiamine deficiency, particularly during the early stages. Although single point measurements of plasma GH levels have limitations in their interpretation, it is clear that exposing rats to 2 weeks of thiamine deficiency resulted in significant reduction in plasma GH levels, without any significant alteration in circulating concentrations of insulin, glucagon, corticosterone. Unpublished observations from our laboratory have demonstrated that the decrease in GH is a reproducible finding and that the drop seen after a 2-week TD period is significantly larger than that resulting from food restriction alone. GH deficiency has been shown to result in a fall in blood IGF-I and IGFBP-3 levels.[16] Conversely, increased secretion of GH is generally associated with increased levels of IGF-I and IGFBP-3.[17,18] Part of the GH-dependent increase in IGFBP-3, however, may be mediated by IGF-I itself, since IGF-I administration has been shown to increase IGFBP-3 concentrations.[19] In contrast, IGFBP-1 and -2 are considered to be GH-independent and are regulated by the prevailing insulin and glucocorticoid levels.[20]

Studies have shown that the insulinopenia of diabetes is associated with marked reductions in tissue and plasma levels of IGF-I and in the plasma levels of IGFBP-3, whereas the levels of IGFBP-1 and -2 are increased. Circulating and tissue levels of IGF-I, as well as the levels of IGFBP-1, -2 and -3 are restored to normal after the animals are treated with insulin.[21] This inverse relationship of IGFBP-1 and -2 to insulin levels appears to contrast that observed in the present study. The drop in IGFBP-1 levels was quite marked at 2 weeks when plasma insulin levels were not depressed. In addition, it is clear that thiamine deficiency was associated with a fall in the IGFBP with a M_r of ≈28,000. This band likely represents one or more of the smaller IGFBPs (IGFBP-4, -5, and/or -6); however it was not further characterized. Taken together, these findings suggest that the regulation of IGF-I and the IGFBPs are uniquely modulated during thiamine deficiency, and that this modulation is distinctly different from any other models of nutrient deprivation and fasting, where the regulation of IGF-I or its binding proteins is highly dependent on either energy availability, substrate delivery or the prevailing hormonal milieu.

During the later stages of thiamine deficiency (6 weeks), several hormonal changes, singly or in combination, could be operative. This period was associated with marked reductions in plasma GH and insulin, and significant elevations in plasma glucagon and corticosterone.[4] GH deficiency alone would account for the drop in circulating and tissue levels of IGF-I and for the drop in IGFBP-3. Similarly, the same changes could be attributed to the prevailing insulinopenia; but this would not account for the simultaneous drop in plasma of IGFBP-1 and -2. The elevated corticosterone levels could also account for the fall in IGF-I, but not the reduction in IGFBP-1 and/or -2.

In summary, the alterations of IGF-I and the IGFBPs induced by thiamine deficiency were generalized to all tissues examined. Based on these findings we can speculate that the regulation of IGF-I synthesis and its biologic actions are dependent on intact energy metabolism at the cellular level. Therefore, lack of cofactor availability for the complete oxidation of glucose to CO_2 and H_2O, such as that observed during thiamine deficiency,[22,23] would result in impaired IGF-I synthesis. Although we did not address the reversibility of the thiamine-deficient effects on the GH-IGF axis, one could predict that restoring tissue and blood levels of thiamine would result in complete reversal of the observed effects. This issue will be addressed in future studies. Understanding the mechanisms by which specific micronutrients control IGF synthesis should permit the formulation of strategies for treatment of specific nutritional deficiencies. This should enable development of better methods for nutritional repletion and wiser uses of anabolic agents in the treatment of these conditions.

Acknowledgments

The authors would like to thank Ms. Dawn Sasvary and Mr. Michael Mazza for their excellent technical assistance. Supported by SUNY BRSG, PHS #B933, DK42562, GM38032.

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