

Original Research

The Influence of Nutrition on IGF-1 in Tube-Fed Profoundly Retarded Adults

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Key words: insulin-like growth factor-I, plasma amino acids, profoundly retarded, tryptophan, zinc

Objective: This study was conducted to determine whether IGF-1 concentrations are low in nonambulant profoundly retarded adults and to identify associated nutritional factors.

Methods: Serum IGF-1, albumin, pre-albumin, creatinine, zinc (Zn) and plasma amino acids were measured before and after a four-week 25% increase in formula in 25 individuals, divided into those fed by day (Group A) or by night (Group B).

Results: Circulating IGF-1 was low in nine of the 22 subjects (40.9%) included in the analysis. Mean IGF-1 increased 10.4% ($p=0.004$). Despite high intakes of essential amino acids and Zn, initial mean plasma tryptophan and phenylalanine were low, and serum Zn was low in 40.9% of subjects. Plasma tryptophan was low at both samplings and correlated with circulating IGF-1 concentrations ($p=0.02$) at the beginning of the study. Serum IGF-1 and Zn also correlated ($p=0.02$) initially.

Conclusions: IGF-1 is commonly low in this population and is associated with low plasma amino acid and Zn concentrations, despite high intakes of these nutrients. The causes and clinical implications of these abnormalities need further study.

INTRODUCTION

Circulating insulin-like growth factor-1 (IGF-1), an anabolic hormone, is sensitive to nutritional status and correlates with accepted biochemical indices, such as serum albumin and prealbumin [1]. IGF-1 concentrations fall during fasting and normalize with refeeding [2].

Low circulating IGF-1 concentrations are associated with adverse health effects, including cognitive function [3], adolescent bone mass acquisition and adult bone integrity [4]. In a hospitalized elderly population, circulating IGF-1 predicted life-threatening infectious and non-infectious complications with 76% accuracy [5].

There are approximately 135,000 people in the U.S. with severe or profound mental retardation [6]. They are prone to a variety of health problems apart from their cognitive limitations, including osteoporosis [7] and premature death [8], the latter often associated with complications of infectious disease. A subset of people with profound mental retardation requires long-term gas-

trostomy tube feedings, thereby making it feasible to determine nutrient intake accurately and optimize nutritional status. It may be possible, by manipulating nutritional intake such that IGF-1 concentrations rise, to realize improvements in those areas where IGF-1 have been shown to affect health.

IGF-1 abnormalities among people with severe disabilities have been described in only one report [9]. In this study seven of ten children with short stature and cerebral palsy had low IGF-1 concentrations. To our knowledge, IGF-1 has not been measured in nonambulant adults with profound mental retardation. We conducted a four-week study to measure circulating IGF-1 concentrations and to evaluate the effects of increased formula intake on IGF-1, Zn, albumin, prealbumin and plasma amino acids. The relationship between the intakes of amino acids and Zn and their blood concentrations was also examined.

Clinical application of the information obtained from this study may permit nutritional manipulations aimed at improving cognition, bone integrity and immune function via optimization of IGF-1 in this population.

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METHODS

Patients

Twenty-five nonambulant, profoundly retarded, metabolically stable, but severely immobile tube-fed adults at Central Wisconsin Center (CWC) were enrolled in the study. Based upon the criteria that follow, the parents or guardians of all eligible adults who were residing at Central Wisconsin Center were invited to participate through an informed consent process. The project was approved by the Central Wisconsin Center Institutional Review Board and the Human Subjects Committee of the University of Wisconsin-Madison.

Subjects were fed either Osmolite HN® or Jevity® (Ross Laboratories, Columbus, Ohio) during this study. These formulas are identical, except for largely insoluble (94%) fiber and additional calcium, phosphorus and chloride found in Jevity. Those who routinely received Osmolite HN or Jevity prior to the study continued on these products while the remaining subjects were switched to these products from similar commercial formulas at least one week prior to the study. Each individual had unique neurological deficits and individualized medication requirements, making it necessary for each subject to act as his or her own control. All formula, water, and medications had been provided exclusively by gastrostomy tube at least one year. All subjects had stable weights. Each received quantities of formula that were consistent with accepted recommendations for optimizing weight, yet were just below intakes empirically found to cause excessive weight gain, based upon ongoing individualized evaluations by staff dietitians.

Children and adolescents were excluded in order to avoid the hormonal and nutritional factors involving growth that may complicate interpretation of IGF-1 and amino acid patterns. In addition, individuals with the following conditions were excluded from the study: known gut absorptive defects (such as gluten-sensitive enteropathy and inflammatory bowel disease), diabetes mellitus, compromised renal function, liver disease, steroid therapy, HIV infection, cancer or identified inborn errors of metabolism.

During the week prior to blood sampling, all subjects received Osmolite HN or Jevity at their customary volumes. A 25% increase in the volume of formula was then introduced in two steps, first by increasing the daily volume by 15% for one week and then by an additional 10% for the final three weeks. (These incremental changes were used to minimize the risk of inducing gastroesophageal reflux.) As the volume of formula was increased, the volume of water was correspondingly decreased. All fluid volumes were carefully recorded by 10 mL increments. In order to meet minimum protein requirements, three subjects received additional protein supplementation with Promod® (Ross Laboratories, Columbus, Ohio). Body lengths (taking into account hip and knee contractures, but not scoliosis) and weights were obtained at the start of the project, and weights were measured weekly.

Subjects were divided into two Groups (Table 1), based solely on their pre-existing feeding schedules: Group A routinely received feedings several times during the day. Group B routinely received formula between 1800 hours and 0600 hours in order to accommodate daytime activity schedules, with the majority given between midnight and 0400 hours (Table 1). Blood specimens were drawn after ten-hour fasts just prior to the first increase in formula and on the last day of the 25% increase in formula intake. The ten-hour fast required that Group A samples be drawn at 0700 hours; Group B samples were collected at 1400 hours.

Serum albumin and creatinine were assayed at the Mendota Mental Health Institute Laboratory, Madison, Wisconsin. Serum prealbumin, C-reactive protein, thyroid stimulating hormone (TSH) and Zn were measured at the Clinical Chemistry Laboratory, University of Wisconsin Hospital and Clinics, Madison, Wisconsin. Serum IGF-1 was measured by Endocrine Sciences, Calabasas Hills, California. IGF-1 was measured by RIA, consistent with the recommendations of Hintz *et al.* [10]. The IGF-1 intra-assay and inter-assay coefficients of variance were 5.4% and 7.3%, respectively. Heparinized blood was chilled immediately after collection, and the plasma was isolated within 30 minutes. These specimens were immediately

Table 1. Characteristics of Study Population (Mean±SEM)

	Group A		Group B	
Males	9		5	
Females	5		3	
Age (years)	23.6±1.3		29.0±2.4	
	Start		End	
	Group A	Group B	Group A	Group B
Body wt (kg)	37.0±1.5	44.0±1.8	38.5±1.3	44.2±1.6
Ht (cm)	146.8±2.0	154.9±3.2	—	—
Intake:				
Kcal/kg	27.6±1.2	26.2±1.7	34.2±1.6	32.3±2.1
Protein (g/kg)	1.19±0.04	1.11±0.06	1.44±0.06	1.36±0.07
Zinc (mg/day)	17.9±1.1	22.1±1.3	20.0±2.6	24.5±2.9

frozen and shipped on dry ice to Ross Laboratories, Columbus, Ohio, for amino acid analysis.

Statistical Analysis

The plasma amino acid data were summarized statistically by Ross Laboratories. Descriptive statistics, *t* tests, and Pearson correlations were performed using Systat software (Systat, Inc., Evanston, IL). In order to reduce the risk of Type I errors in the context of multiple comparisons, the level of statistical significance was reduced to 0.02. All tests were two-tailed.

RESULTS

Mean IGF-1 concentrations rose 10.4% after four weeks of supplemental formula (Fig. 1, Table 2). Nine of the 22 subjects (40.9%) had low IGF-1 concentrations for age and gender at the start of the study. IGF-1 normalized in one of these nine and increased in five of the remaining eight subjects. IGF-1 concentrations trended higher in Group B (night-fed) than in Group A (day-fed), namely 43.3 ± 8.4 vs. 10 ± 9.9 $\mu\text{g/L}$, (mean \pm SEM, $p=0.03$).

At the start of the study, correlations were found between IGF-1 concentrations and plasma tryptophan ($r=0.49$, $p=0.02$), isoleucine ($r=0.55$, $p=0.01$), leucine ($r=0.48$, $p=0.02$), valine ($r=0.48$, $p=0.02$) and serum Zn ($r=0.50$, $p=0.02$) for the entire group. None of these correlations were found at the end of the study.

Plasma concentrations of several amino acids (Table 3) were low compared to a group of normal people receiving similar amounts of protein, i.e., graduate students ingesting one gram/kg body weight/day. (Ross Laboratories, unpublished data) Most noteworthy were the initial low concentrations of

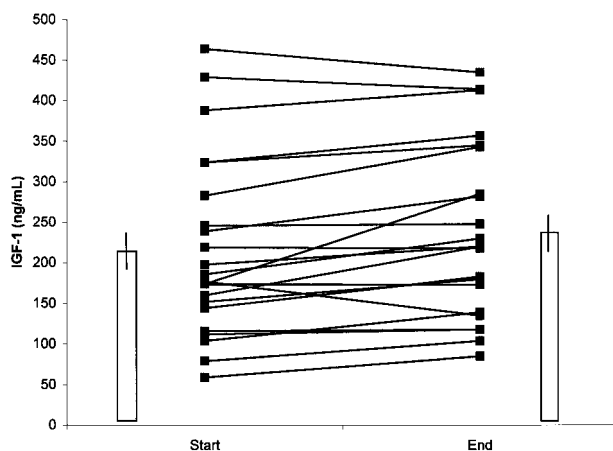


Fig. 1. Combination graphic of serum IGF-1 concentrations, with mean \pm SEM (vertical bars) and each individual's data points at the start and end of study ($n=22$).

tryptophan and phenylalanine in our study group. Following the supplemental formula, valine and phenylalanine increased, glutamine, glycine and threonine decreased, and tryptophan did not change. Although our standard for statistical significance was not reached, plasma tryptophan concentrations were marginally higher for Group A (day-fed) than Group B (night-fed) in both pre-supplementation (50.0 ± 2.6 vs. 40.4 ± 3.5 $\mu\text{mol/L}$, mean \pm SEM, $p=0.05$) and end-supplementation samplings (49.4 ± 2.5 vs. 41.2 ± 2.6 , $p=0.04$). There were no differences in the other amino acid concentrations between Groups A and B.

The initial ratios of mean cystine/glycine, tyrosine/glycine, and valine/glycine concentrations were low but all three ratios rose significantly ($p<0.001$) after ingestion of the additional formula.

Nine subjects (40.9%), two of whom received valproic acid routinely, had serum Zn concentrations below the normal range (10.7 – 18.4 $\mu\text{mol/L}$) at the start of the study, and seven subjects had low concentrations at the conclusion of the study. However, there was no overall change after receiving the additional formula.

Prealbumin, although normal in both samplings, showed a significant increase at the end of the project ($p<0.001$).

Serum albumin concentrations were normal in both samplings, did not change during the study and initially correlated with plasma tryptophan ($r=0.65$, $p=0.001$) and serum Zn ($r=0.62$, $p=0.002$). At the conclusion of the study the correlation of albumin with tryptophan ($r=0.51$, $p=0.01$) and Zn persisted ($r=0.76$, $p<0.001$).

Slightly elevated C-reactive protein concentrations were noted in two subjects initially, one of whom had low IGF-1 that improved following the additional formula. A third individual had slightly elevated C-reactive protein at the end of the study. Although eight subjects received antibiotics for minor infections, these findings show that neither infection nor inflammation appeared to complicate interpretation of the data. Since all thyroid-stimulating hormone values were normal, none of the IGF-1 or amino acid abnormalities could be attributed to abnormal thyroid status.

Two subjects did not complete the study. One experienced vomiting believed to be due to severe constipation and required intravenous fluids. The other developed a severe urinary tract infection near the end of the study. Each of these individuals required temporary changes in intake judged likely to make analyses unreliable. Based upon extremely low initial and undetectable final IGF-1 concentrations, an abnormal sella turcica, and suspected hypopituitarism, the data from a third individual were excluded from analysis. No changes in routine medications were made except for minor alterations in those used to relieve constipation. Nineteen of the 22 people included in the analysis received anticonvulsants, with two receiving valproic acid. Circumstantially, 11 individuals received Osmolite HN, and 11 received Jevity.

Table 2. Serum Analyses (Mean±SEM) Measured at Start and End of the Study

Assay	Normals	Start	p	End
Albumin (g/L)	32.0–52.0	41.6±0.7	ns	42.5±0.6
Prealbumin (mg/dL)	15–29	21.9±1.0	<0.001	24.4±0.9
Creatinine (μmol/L)	44.2–106.1	65.5±1.9	0.002	60.3±2.17
Zinc (μmol/L)	10.71–18.35	11.13±0.34	ns	11.27±0.21
TSH (mU/L)	0.30–4.30	1.27±0.14	—	—
IGF-1 (μg/L)	Age/Gender-Dependent	216.0±23.8	0.004	238.4±22.9

Table 3. Plasma Amino Acid Values (μmol/L, Mean±SEM) and p Values at Start and End of Study, with Reference Values Comparable to this Setting (Graduate Students Receiving 1 g/kg/day Protein, Provided by Ross Laboratories)

Amino Acid	Reference Values	Start	p	End
Methionine	33.5±2.2	23.7±0.6	ns	23.2±0.8
Valine	230.5±13.1	177.6±5.2	0.011	192.7±6.0
Leucine	124.0±12.7	98.2±3.7	ns	103.6±4.1
Isoleucine	62.2±5.8	54.1±2.4	ns	57.4±2.3
Threonine	141.6±7.0	152.8±10.1	ns	136.1±7.0
Lysine	199.2±12.1	207.5±10.0	ns	200.4±8.1
Phenylalanine	61.4±4.3	30.0±0.8	<0.001	34.1±1.0
Histidine	79.9±4.3	90.4±1.7	ns	91.3±2.1
Arginine	113.3±9.4	88.0±4.4	ns	82.4±3.9
Alanine	504.7±44.4	367.3±15.0	ns	384.4±18.0
Glycine	285.8±11.8	292.0±10.2	0.003	264.0±11.1
Serine	119.3±6.7	115.3±4.9	ns	107.8±5.8
Tyrosine	70.9±6.1	59.6±1.7	ns	60.9±2.3
Proline	227.9±15.5	201.3±17.2	ns	206.4±14.7
Glutamine	660.9±33.8	773.7±24.1	0.002	718.8±23.2
Glutamic acid	22.4±4.2	45.1±4.3	ns	41.3±3.1
Asparagine	54.8±2.4	49.5±1.4	ns	48.6±1.7
Aspartic Acid	3.7±0.3	4.3±0.3	ns	4.1±0.2
Cystine	52.4±3.3	61.4±1.4	ns	62.2±1.7
Ornithine	52.4±3.3	43.3±2.2	ns	43.2±1.8
Citrulline	46.2±5.3	29.6±1.5	ns	31.9±1.5
Tryptophan	73.5±2.8	46.5±2.4	ns	46.4±2.1

DISCUSSION

This study documents low concentrations of serum IGF-1, certain plasma amino acids and serum Zn among formula-fed nonambulant individuals receiving recommended amounts of formula. The short-term increase in formula was associated with improvement in IGF-1, suggesting that a relative deficiency of a nutritional component contributed to low IGF-1 status. IGF-1, which normally shows little or no diurnal variation [1], responded marginally better in individuals fed at night.

The importance of the availability of amino acids for the synthesis of IGF-1 has been documented by *in vitro* hepatocyte studies. Tryptophan is particularly crucial for protein synthesis, as observed by Harp *et al.* [11], who found significant declines in hepatic IGF-1 mRNA levels and IGF-1 release in culture media that are devoid of tryptophan or lysine. Supplementation with tryptophan to half-normal plasma concentrations raised IGF-1 concentrations to 78% of those from cells in complete media. Similarly, Brameld, *et al.* [12], using a pig hepatocyte

culture system, showed dramatic dose-dependent reductions in IGF-1 mRNA synthesis involving arginine, proline, threonine, tryptophan and valine. An amino acid-responsive element that is particularly tryptophan-sensitive has been identified in the rat IGF-1 gene [12].

Plasma tryptophan is especially noteworthy in our study because it was low at both samplings and it correlated with circulating IGF-1 concentrations at the beginning of the study. Low plasma tryptophan was also found in a large sample of institutionalized, severely and profoundly retarded adults [13].

The plasma amino acid picture in our study was otherwise mixed, with features of both malnutrition and adequate intake. On one hand, the low concentrations of most essential amino acids (isoleucine, leucine, methionine, phenylalanine, tryptophan and valine) are consistent with malnutrition [14]. The improvement in the ratios of cystine/glycine, tyrosine/glycine and valine/glycine was also consistent with some degree of malnutrition [15]. On the other hand, normal albumin, prealbumin and glycine and low alanine concentrations were not typical of malnutrition [14]. Our results are consistent with a

Table 4. Amino Acid Intakes (mg/kg, Mean±SEM), Prior to Formula Increase, Compared to Two Established Recommendations

	Intake	Range	1985 FAO/WHO/UNU Requirements	Revised Estimates (Young and El-Khoury)
Isoleucine	51.9±1.6	33.6–62.8	10	23
Leucine	103.8±3.3	66.5–128.6	14	39
Lysine	82.6±2.5	54.0–102.8	12	30
Methionine+Cystine	36.3±1.1	24.2–44.3	13	15
Phenylalanine+Tyrosine	112.1±4.0	67.8–142.8	14	39
Threonine	48.9±1.5	33.0–66.0	7	15
Tryptophan	13.5±0.4	8.8–16.6	3.5	6
Valine	63.6±2.0	40.5–80.0	10	20

degree of hypoaminoacidemia, whether due to malabsorption or abnormal flux, despite normal total protein and high essential amino acid intakes. Except for tryptophan, the plasma amino acid pattern we observed is similar to that of formula-fed elderly men receiving similar quantities of protein (0.8–1.2 g/kg) that provided essential amino acids 1.0 to 2.9 times higher than the RDA [16]. Protein intake has a complicated effect on plasma amino acid patterns, depending upon a number of factors. In contrast to our findings, Forslund *et al.* [17] demonstrated a prominent inverse relationship between protein intake and plasma amino acids in healthy men ingesting 1 g/kg vs. 2.5 g/kg.

It is important to emphasize that the plasma essential amino acid concentrations observed in this study were neither due to inadequate intake of these nutrients (Table 4) nor the result of circadian change [18]. Even prior to receiving the supplemental formula, essential amino acids intakes were up to eight times the recommended intakes of the 1985 FAO/WHO/UNU Expert Consultation and more than twice the recent recommendations of Young and El-Khoury [19].

Similarly, Zn intakes were above the currently recommended intakes of 12 mg (adult female) and 15 mg (adult male) per day [20]. Low plasma Zn concentrations were previously found in profoundly retarded individuals, and dietary Zn intakes 50% higher than the recommended reference range were required in order to achieve Zn balance, indicating Zn malabsorption [21].

Zn status alone may explain our observations regarding IGF-1. Roth and Kirchgebner found that the administration of a Zn-deficient diet to rats by gastric tube causes IGF-1 to fall [22]. Others reported that serum growth hormone-binding protein is regulated by dietary Zn, a deficiency of which causes parallel reductions in both growth hormone and serum IGF-1 [23]. In Zn-deficient children with short stature, Zn supplementation increased circulating IGF-1 and induced growth [24]. In a study of postmenopausal women, Zn intake was the major determinant of IGF-1 concentrations among age, body weight and 25 nutrient variables [25].

Based upon a Zn turnover model [26], a longer period of increased Zn intake may be required to observe changes in serum Zn concentrations and to optimize Zn pools. It is acknowledged that serum Zn is imprecise in determining Zn

status. Although alternate methods are being investigated [27], serum Zn is the most commonly used and most readily available method of estimating Zn status. Anticonvulsants, especially sodium valproate, may also modify Zn status [26].

Caloric intake, another determinant of IGF-1, is lower than normal in people with severe developmental disabilities. It is therefore necessary to balance the risks of providing inadequate calories with the risks of obesity. Formulas for determining caloric requirements are imprecise when applied to individuals, and adjustments, based upon weight responses, are the primary way of optimizing intake [28]. It is uncertain whether a longer period of increased formula intake for our subjects would have resulted in further biochemical improvements, but excessive weight gain, based upon the trends observed in this study, would likely have occurred.

CONCLUSION

This study revealed low IGF-1 concentrations in a significant number of nonambulant profoundly retarded adults. Essential amino acid and Zn status, as well as the timing of feedings, may influence IGF-1 concentrations. Confirmation of these observations and investigation of their clinical significance, especially with regard to cognitive function, bone integrity and immune function, is needed.

ACKNOWLEDGMENT

We wish to gratefully acknowledge the financial support and services of Ross Laboratories, Columbus, Ohio.

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Received May 31, 2000; revision accepted December 15, 2000.