

# Evaluation of insulin-like growth factor-1, total ghrelin, and insulin resistance in nutritionally stunted Egyptian children

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## Original Article

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## ABSTRACT

Stunting represents a worldwide nutritional problem, which has many negative effects on child health and development. This study aimed to investigate the levels of insulin-like growth factor-1 (IGF-1), total ghrelin and insulin resistance, as well as certain micronutrients affecting children growth and skeletal maturity in nutritionally stunted Egyptian children. Stunted children were selected to have height for age Z score (HAZ) < -2 standard deviation, whereas, control children were selected to have HAZ > -2. Stunted children were further classified according to weight for age Z score into normal and underweight groups. All stunted children exhibited significantly lower serum levels of IGF-1, Ca, and Mg compared to normal control children. Additionally, both normal weight and underweight groups showed insignificantly higher total ghrelin levels than the control values. Only stunted underweight children showed significantly lower serum Zn levels compared to control children. Meanwhile, no significant differences were observed in serum glucose and insulin levels, pancreatic beta cell function and insulin resistance between different study groups. In conclusion, these findings highlight the importance of early detection of abnormalities in growth hormone / IGF-1 axis and micronutrients levels in hope that appropriate intervention strategies could improve their status to obtain full growth potential in nutritionally stunted Egyptian children.

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**Key Words:** Ghrelin; IGF-1; Insulin resistance; Micronutrient; Stunting.

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## 1. INTRODUCTION

Stunting or short stature is defined as having height-for-age z-score (HAZ) value of less than minus two standard deviations from the WHO Child Growth Standards median<sup>[1]</sup>. It is estimated that about 22.9% of children under five years of age globally<sup>[2]</sup>, and 31% in Egypt<sup>[3]</sup>, are stunted. Stunting is associated with increased morbidity, lower intelligence, reduced physical, neuro-developmental and economic capacity<sup>[4,5]</sup>.

Short stature can be classified into two main types: normal variant and pathological. Normal variant type includes familial and idiopathic short stature (ISS) as well as constitutional delay of growth and puberty (CDGP). Whereas, pathological short stature includes various causes such as malnutrition, genetic, and endocrine diseases<sup>[6]</sup>.

Growth hormone (GH) / insulin like growth factor-1 (IGF-1) axis is an important pathway in the regulation of

linear growth<sup>[7]</sup>. IGF-1 is a hormone similar in molecular structure to proinsulin, and is produced primarily by the liver under the control of GH<sup>[8,9]</sup>. IGF-1 is affected by various factors such as nutrient intake and age<sup>[10]</sup>. One of IGF-1's principal functions is to mediate the growth-promoting effects of GH in peripheral tissues<sup>[11]</sup>. In contrast to pulsatile GH secretion, circulating IGF-1 is stable and reflects the long-term status of GH secretion<sup>[12]</sup>. Therefore, measurement of serum IGF-1 concentration can complement assessment of GH status in the investigation of short stature and contribute to prediction of growth response during GH therapy<sup>[7,13]</sup>.

Ghrelin is another component related to GH/IGF-1 axis. It is a peptide hormone produced mainly in the stomach, and lower amounts can be derived from small intestine, pancreas, kidneys, and other organs<sup>[14]</sup>. Ghrelin was initially identified as the endogenous ligand of the GH secretagogue receptor in the brain, which is capable of

stimulating GH release from the anterior pituitary gland. It acts as natural orexigenic factor and long-term body weight regulator, and has a role in glucose homeostasis, immunity, and cardio protection<sup>[15]</sup>. Consequently, ghrelin could affect growth by stimulating appetite and food intake and by altering GH secretion, subsequently affecting IGF-1 generation. Recently, it has been found that the total amount of fetal ghrelin levels is higher in comparison to the amount in adults which affirms its importance in prenatal life<sup>[16]</sup>. Moreover, it was suggested that ghrelin may serve to stimulate appetite in small fetuses to adapt to intrauterine malnutrition<sup>[17,18]</sup>. Besides, there is accumulating preclinical and clinical evidence supporting a beneficial effect of ghrelin pathway inhibition for the treatment of type 2 diabetes<sup>[19]</sup>.

Malnutrition in early life has been reported to impair the expression of several insulin-signaling proteins, which may represent early markers of insulin resistance and metabolic disease risk<sup>[20]</sup>. However, conflicting results have been reported about the effects of undernutrition during childhood on glucose/insulin metabolism. A recent study demonstrated that the exposure to severe famine in the fetal or childhood period may predict a higher glycated haemoglobin (HbA1c) and increased diabetes risk in adulthood<sup>[21]</sup>. However, other studies demonstrated an insignificant change in glucose/insulin axis in short stature children in South Africa<sup>[22]</sup> and Germany<sup>[23]</sup>.

Furthermore, micronutrient malnutrition is known to be a major health burden in developing countries<sup>[24]</sup>. The child's brain is rapidly growing and has a high demand for energy, protein, essential fats, and key micronutrients, accordingly, deficiencies during this critical period may have long-term and irreversible effects on brain development<sup>[25,26]</sup>. It has been reported that inadequate intakes of zinc (Zn), necessary in cellular replication and immune response development, are associated with impaired growth, diarrhea, delayed sexual maturation, and impaired appetite<sup>[27]</sup>.

Another essential element is calcium (Ca) which is especially important in bone tissue, as it is an essential component of mineralized bone matrix, and cellular calcium signaling is a key process in the formation and repair of bones<sup>[28,29]</sup>.

On the other hand, magnesium (Mg) is a crucial element that is implicated in all metabolic and biochemical processes in the cell, including energy metabolism, DNA replication, protein synthesis, and the control of cell proliferation<sup>[30]</sup>. Moreover, Mg deprivation has been reported to be associated with energetic and oxidative stress in several cell types<sup>[31,32]</sup>. Recently, *Sargenti et al.*<sup>[33]</sup> showed that Mg deprivation modulates stem cell plasticity and differentiation potential.

To the best of our knowledge, little information is available about serum levels of IGF-1 and total ghrelin in nutritionally stunted Egyptian children. Therefore, the present study was undertaken to investigate the possible

changes in the level of these crucial hormones, and to assess insulin levels and pancreatic beta cell function, as well as the levels of certain essential micronutrients in nutritionally stunted Egyptian children in a trial for the early identification of growth abnormalities to prevent their possible harmful effects in the future.

## 2. METHODS

### 2.1 Participants

The study included 87 pre-pubertal children classified according to HAZ into 59 stunted (HAZ < -2 standard deviation score (SDS)), and 28 healthy control (HAZ > -2 SDS) during the period from November 2013 to March 2014. The stunted children (aged from 4 to 10 years) were recruited from the stunting outpatient clinic of the National Nutrition Institute, Cairo, Egypt. The stunted group was further subdivided according to weight for age Z score (WAZ) into two groups, normal weight-stunted (WAZ > -2 SDS), and underweight-stunted children (WAZ < -2 SDS). Children with hypothyroidism and/or suffering from chronic cardiovascular, respiratory or urinary system diseases, as well as children with complaints of the gastrointestinal tract were excluded from the study. The control group was selected from healthy children aged from 4.9 to 9.5 years. They were chosen to have normal weight and height, as well as normal history of growth and development.

The stunted children and the control ones were subjected to full history including age, sex, previous diseases, operations, history of drug intake. Weight was measured to the nearest 0.1 kg using an electronic digital scale, and height was measured to the nearest 0.1 cm using a stadiometer, then the body mass index (BMI) was calculated using the following formula: BMI = weight (kg)/height (m)<sup>2</sup>. HAZ, WAZ, and BMI-for-age Z-scores (BMIZ) were calculated for each child with the use of World Health Organization's AnthroPlus software<sup>[34]</sup>. The Clinical examination was performed by a physician and routine investigation including complete blood picture, urine and stool analyses were done. The study was conducted according to the Declaration of Helsinki Principles, and was approved by the Research Ethics Committee for Experimental and Clinical Studies of the Faculty of Pharmacy, Cairo University (approval number BC 777), and by that of the National Organization for Teaching Hospitals and Institutes (approval number IN000065). Written informed consent was obtained from the parents of each participant after the nature of the procedures had been explained.

### 2.2 Biochemical analyses

Five milliliters of blood were collected in a clean tube after 12h of overnight fasting. Serum was obtained by centrifugation at 3000 rpm for 15 min in a refrigerated centrifuge. Samples were processed as quickly as possible and kept on ice. The separated sera were divided into five portions. One was immediately used to determine serum

glucose level, and the other four portions were stored at  $-20^{\circ}\text{C}$  for subsequent laboratory investigations.

### 2.2.1 Estimation of IGF-1, total ghrelin and insulin

IGF-1 was measured by solid phase competitive ELISA kit (DRG International inc., USA), while total ghrelin and insulin were measured by solid phase sandwich ELISA kits (Glory Science Co., Ltd, USA, and DRG International inc., USA, respectively) according to the manufacturers' instructions.

### 2.2.2 Estimation of glucose

Glucose was measured by colorimetric enzyme method described by Trinder<sup>[35]</sup> using Stanbio laboratory kit (USA).

### 2.2.3 Assessment of pancreatic beta cell function and insulin resistance

The homeostasis model assessment version 2 (HOMA2) was used to evaluate pancreatic beta cell function (HOMA2-%B) and insulin resistance (HOMA2-IR) from the paired fasting glucose and insulin concentrations using HOMA2 calculator version 2.2<sup>[36]</sup>. Results were expressed as a percentage of the normal reference population.

### 2.2.4 Estimation of Ca, Mg, and Zn

Ca level was determined according to the method of Gindler and King<sup>[37]</sup> using Biosystems S.A. kit (Spain). Mg level was measured according to the method of Mann and Yoe<sup>[38]</sup> using Spectrum Diagnostics kit (Egypt). Zn level was measured according to the method of Homsher and Zak<sup>[39]</sup> using Quimica Clinica Aplicada kit (Spain).

### 2.3 Statistical analyses

The data were analyzed using the computer based statistical package of statistical product and service solutions (SPSS version 20.0, SPSS Inc. Chicago, IL, USA). All the data are expressed as mean  $\pm$  standard error of mean (SEM). One way ANOVA (post hoc; Tuckey test) was applied in the evaluation of normally distributed variables. Non parametric Kruskal–Wallis test was used when necessary. The relationship between IGF-1, ghrelin, and minerals with different measured parameters were done using the bivariate correlation by the use of Spearman correlation coefficient. *P*-value of  $< 0.05$  was considered significant. Multiple regression analyses were done with standardized coefficients and values of  $P < 0.05$  were considered statistically significant.

## 3. RESULTS

### 3.1 Anthropometric characteristics

Anthropometric characteristics of all groups were summarized in (Table 1). There was no significant difference in age between different studied groups. Stunted

normal and underweight children exhibited significantly lower weight, height, WAZ and HAZ compared to control children. Stunted normal weight children showed significantly higher BMI and BMIZ, while stunted underweight children showed significantly lower values for these two parameters compared to control values. Moreover, stunted underweight children had significantly lower WAZ, BMI, and BMIZ compared to the stunted normal weight children.

**Table 1:** Anthropometric characteristics and minerals levels of different study groups

Parameters	Control children (n=28)	Stunted normal weight children (n=29)	Stunted underweight children (n=30)
Age (years)	7 $\pm$ 0.3 (4.9–9.5)	6.8 $\pm$ 0.4 (4.2–10)	7.1 $\pm$ 0.3 (4–10)
Sex (M/F)	(15/13)	(14/15)	(13/17)
Weight (kg)	21.4 $\pm$ 0.9 (15.5–32.8)	18.6 $\pm$ 0.7* (14–26)	16.3 $\pm$ 0.6** (11.5–21)
Height (cm)	116.5 $\pm$ 1.9 (101.5–136.2)	103.4 $\pm$ 1.53* (91.5–121)	104.8 $\pm$ 1.71* (87.7–119.5)
WAZ	-0.6 $\pm$ 0.1 (- 1.8–0.8)	-1.4 $\pm$ 0.1* (-1.97–0.08)	-2.6 $\pm$ 0.1** (- 3.8– - 2.1)
HAZ	-0.9 $\pm$ 0.2 (-1.98–1.73)	-2.9 $\pm$ 0.1* (- 4.2– -2.2)	-3.2 $\pm$ 0.1* (- 4.7– -2.3)
BMI (kg/m <sup>2</sup> )	15.5 $\pm$ 0.2 (14–17.7)	16.8 $\pm$ 0.2* (14.9–19.6)	14.8 $\pm$ 0.1** (12.9–16.7)
BMIZ	-0.1 $\pm$ 0.1 (- 1.3–0.8)	0.7 $\pm$ 0.1* (- 0.5–2.2)	-0.6 $\pm$ 0.1** (- 2.3–0.5)
Ca (mmol/l)	2.6 $\pm$ 0.02 (2.3–2.8)	2.2 $\pm$ 0.04* (1.9–2.6)	2.3 $\pm$ 0.04** (1.8–2.7)
Mg (mmol/l)	1.3 $\pm$ 0.04 (0.8–1.6)	0.8 $\pm$ 0.07* (0.3–1.5)	0.9 $\pm$ 0.05* (0.3–1.5)
Zn ( $\mu\text{mol/l}$ )	25.6 $\pm$ 0.2 (23.4–28.1)	24.5 $\pm$ 0.7 (14.3–28.9)	23.1 $\pm$ 0.5** (14.2–28.3)

Data are expressed as mean  $\pm$  SEM, range is stated between parentheses.

BMI, body mass index; BMIZ, body mass index-for-age Z score; HAZ, height-for-age Z score; WAZ: weight-for-age Z score; Ca, calcium; Mg, magnesium; Zn, zinc.

\*Significant difference from control group at  $P < 0.05$ , \*\* significant difference from stunted normal weight group at  $P < 0.05$ .

### 3.2 IGF-1 and total ghrelin levels

As shown in (Figure 1), stunted normal and underweight children showed significantly lower IGF-1 levels than control children (138.7 $\pm$ 11.7 ng/ml, 118.9 $\pm$ 9.4 ng/ml, 182.2 $\pm$ 15.1 ng/ml, respectively). On the other hand, results presented in (Figure 2) indicated that serum total ghrelin levels of the stunted normal and underweight children were insignificantly higher than those of control ones (1244.5 $\pm$  124.2 ng/l, 1317.7  $\pm$  146.9 ng/l, 1046.4  $\pm$  91.1 ng/l, respectively).

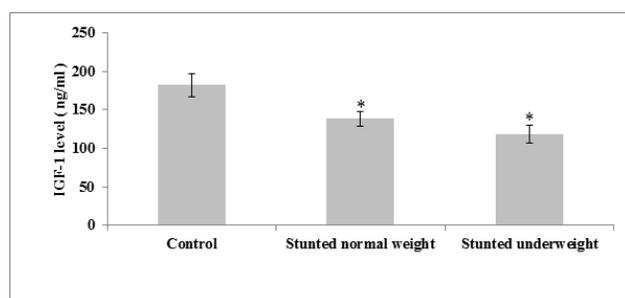


Fig. 1: Serum IGF-1 levels in different studied groups. Data are expressed as mean±SEM. \* Significant difference from the control group at  $p < 0.05$

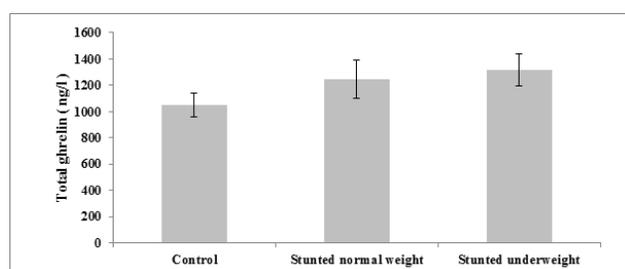


Fig. 2: Serum total ghrelin levels in different studied groups. Data are expressed as mean±SEM

### 3.3 Pancreatic beta cell function and insulin resistance:

Data presented in (Figure 3) indicated that there were no significant differences in the serum levels of glucose (Figure 3a), insulin (Figure 3b), insulin resistance (Figure 3c) and pancreatic beta cell function (Figure 3d) in different studied groups.

### 3.4 Ca, Mg, and Zn levels:

Stunted normal and underweight children exhibited significantly lower Ca (Figure 4a) and Mg (Figure 4b) levels compared to control group. Furthermore, serum Ca levels of stunted underweight children were significantly higher than those of stunted normal weight (Figure 4a). Meanwhile, serum Zn levels of stunted underweight children were significantly lower compared to stunted normal weight and control children (Figure 4c).

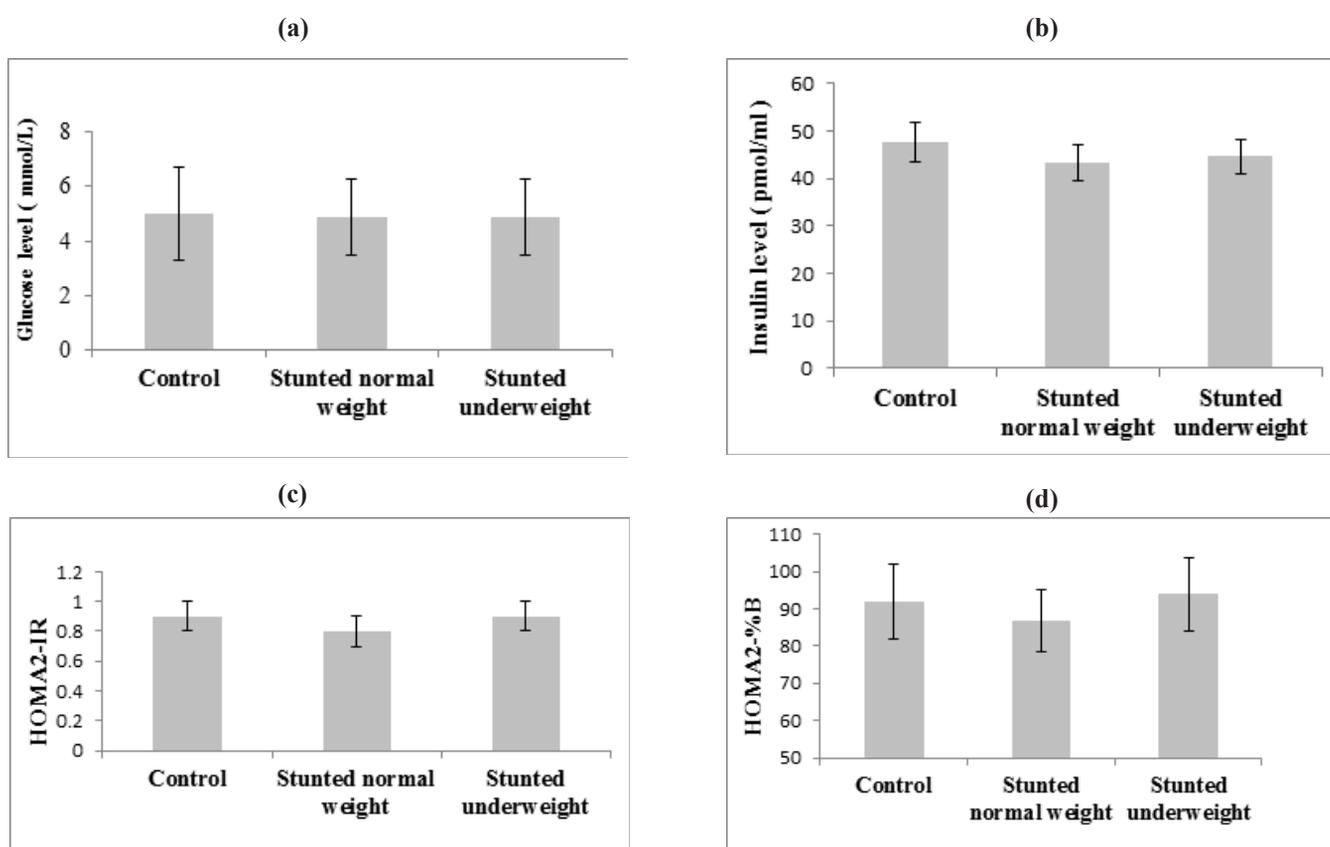
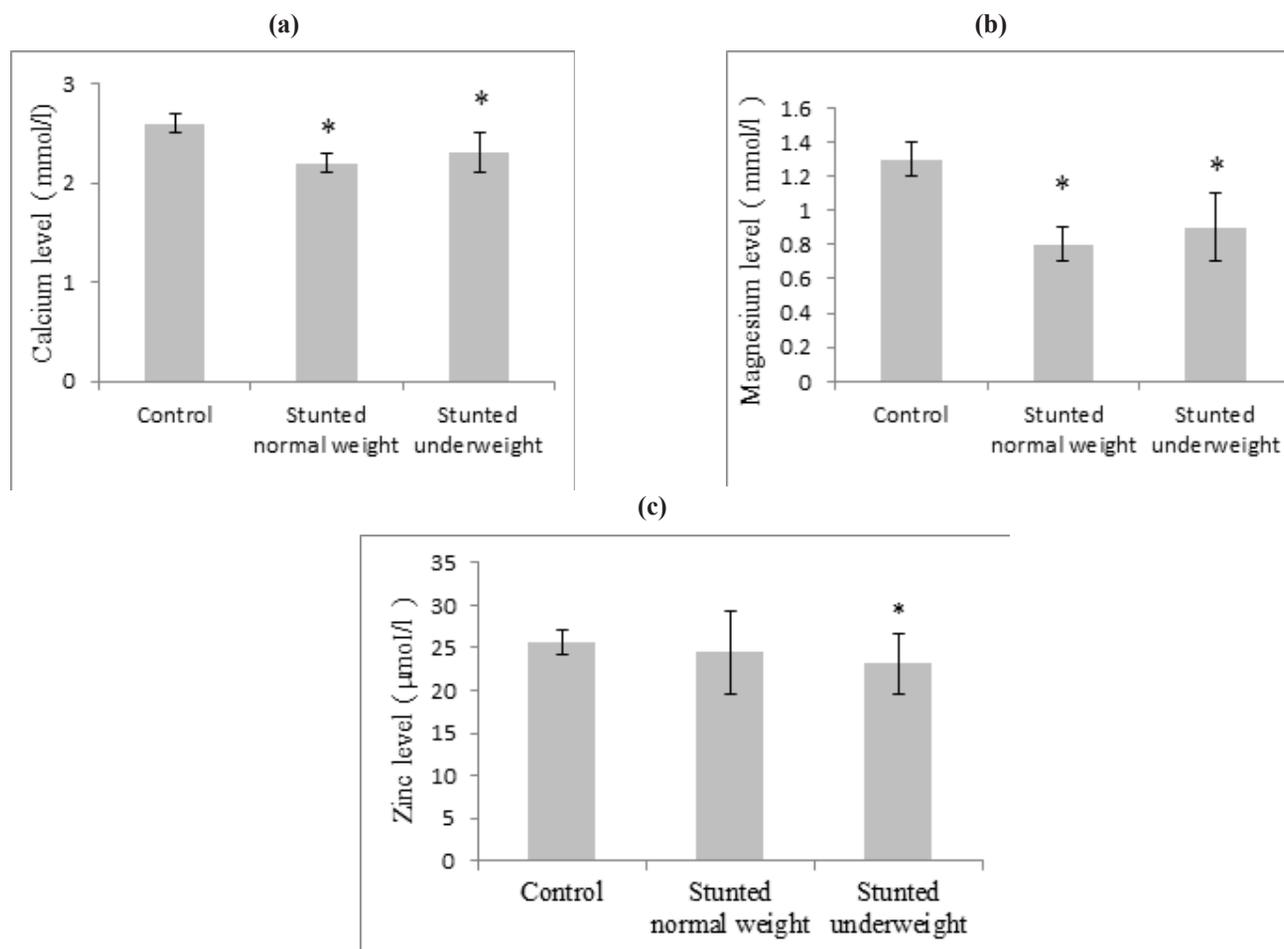


Fig. 3: Glucose- insulin axis in different studied groups.  
 (a) Serum glucose levels  
 (b) Serum insulin levels  
 (c) homeostasis model assessment of insulin resistance (HOMA2-IR)  
 (d) homeostasis model assessment of pancreatic beta cell function (HOMA2-%B)



**Fig. 4:** Serum minerals levels in different studied groups.

(a) Serum Ca levels

(b) Serum Mg levels

(c) Serum Zn levels

\* Significant difference from the control group at  $p < 0.05$

### 3.5 Correlation of IGF-1, total ghrelin, and micronutrients levels with the anthropometric and biochemical parameters:

Results in (Table 2) showed that IGF-1 exhibited significant positive correlation with age, weight, and height in both stunted groups. However, IGF-1 showed significant positive correlation with HAZ in stunted

underweight children only. No significant correlations were detected between total ghrelin and any of the studied anthropometric or biochemical variables in the two stunted groups. In stunted normal weight children, serum Mg showed significant negative correlation with glucose (correlation coefficient ( $r$ ) = -0.4,  $p$  value=0.03). While, serum Zn and Ca did not show significant correlations with any of the measured parameters.

**Table 2:** Correlation between IGF-1 and the measured parameters in the stunted groups

Parameters	Stunted normal weight children		Stunted underweight children	
	Correlation coefficient (r =)	P-value	Correlation coefficient (r =)	P-value
Age	0.530	0.004*	0.611	<0.001*
Weight	0.477	0.010*	0.645	<0.001*
Height	0.469	0.012*	0.685	<0.001*
BMI	0.057	0.773	- 0.023	0.903
WAZ	- 0.077	0.699	0.167	0.378
HAZ	- 0.136	0.491	0.449	0.013*
BMIZ	- 0.135	0.493	- 0.326	0.078
Glucose	0.081	0.682	- 0.184	0.330
Insulin	0.186	0.344	0.072	0.706
HOMA2-%B	0.082	0.674	0.100	0.599
HOMA2-IR	0.337	0.074	- 0.111	0.558
Total ghrelin	- 0.090	0.649	- 0.055	0.773
Zn	0.250	0.191	0.101	0.596
Ca	0.020	0.920	0.233	0.215
Mg	0.165	0.393	0.148	0.435

\* Significant correlation at  $p < 0.05$

BMI, body mass index; BMIZ, body mass index-for-age Z score; Ca, calcium; HAZ, height-for-age Z score; HOMA2-%B, homeostasis model assessment of pancreatic beta cell function; HOMA2-IR, homeostasis model assessment of insulin resistance; Mg, magnesium; WAZ, weight-for-age Z score; Zn, zinc.

### 3.6 Multiple regression of IGF-1 with the measured parameters in the stunted groups

On performing multiple regression, it was demonstrated that height is a significant predictor of IGF-I in the stunted underweight children group only ( $\beta = -8.38$ ,  $p = 0.022$ ). No significant predictors were observed in the stunted normal weight group.

## 4. DISCUSSION

The main findings of the current study indicated that stunted Egyptian children, both normal and underweight, exhibited significantly lower levels of serum IGF-1 along with insignificantly higher total ghrelin levels. No change was observed in serum levels of glucose and insulin, beta cell function, and insulin resistance in both stunted children groups compared to the control ones. Moreover, normal and underweight stunted children demonstrated significantly lower levels of serum Ca and Mg, whereas only stunted underweight children showed significantly lower serum Zn levels.

One of the most evident consequences of undernutrition is the restriction of linear growth, which is mainly a result of GH-IGF-I axis disruption<sup>[40]</sup>. In the present study, nutritionally stunted Egyptian children showed significantly lower serum IGF-1 levels. This observation is in harmony with the study of *Metwalley et al.*<sup>[41]</sup> in which Egyptian children with short stature and GH deficiency exhibited

significantly lower IGF-1 levels. It also agreed with the work of *Hamza et al.*<sup>[42]</sup> who demonstrated that serum IGF-1 levels were significantly low in short Egyptian children with Zn deficiency.

Our results also in accordance with the decreased IGF-1 levels in the serum of French pre-pubertal children with ISS<sup>[43]</sup> and the reported lower IGF-1 levels in severely stunted undernourished Brazilian children compared to moderately stunted children<sup>[44]</sup>. Likewise, *Stawerska et al.*<sup>[45]</sup> found that IGF-1 levels were significantly lower in short thin Polish children with ISS compared to the control group. On the same line, *DeBoer et al.*<sup>[46]</sup> reported insignificantly lower serum IGF-1 levels in stunted malnourished Brazilian children compared to non-stunted counterparts.

In fact, GH-driven linear growth can only occur when GH can induce IGF-1 and other paracrine growth factors as a result of the appropriate dietary signaling from key nutrients, such as essential amino acids and Zn<sup>[47]</sup>. Besides, it has been suggested that decreased plasma IGF-1 levels during the periods of undernutrition may be a part of the body's effort to adapt by shunting calories away from nonessential processes including growth<sup>[48]</sup>. Moreover, undernutrition can affect linear growth by various mechanisms including reduction of GH and IGF-1 receptors<sup>[49]</sup> and increasing fibroblast growth factor 21 levels that inhibit GH binding and action in the epiphyseal growth plate<sup>[50]</sup>. Additionally, malnutrition induces sirtuin-1, a nutritionally responsive protein, that blocks the JAK/STAT pathway and inhibits GH signal transduction in the liver<sup>[51]</sup>. Furthermore, it has been shown that malnutrition causes a reduction in microRNA-21 that promotes chondrocyte proliferation and bone matrix synthesis<sup>[52]</sup>. Interestingly, increased levels of IGF-1 and HAZ were observed in stunted Brazilian pre-pubertal children after nutritional treatment<sup>[53]</sup>.

In the current study, IGF-1 showed significant positive correlations with age, weight and height in both stunted children groups and with HAZ in stunted underweight children. These observations are in harmony with the study of *Camurdan et al.*<sup>[54]</sup> which demonstrated that serum IGF-1 values were significantly correlated with age, weight, height, and height SDS in Turkish children with familial short stature. Similarly, *DeBoer et al.*<sup>[46]</sup> reported that IGF-1 was positively associated with HAZ and WAZ in Brazilian malnourished children. Furthermore, *Wang et al.*<sup>[55]</sup> demonstrated a significant positive correlations between the level of IGF-1, height, weight, and age in Chinese children with ISS.

Ghrelin is a potent stimulator of GH secretion from the pituitary<sup>[56,57]</sup>, and it has been found to be the most powerful orexigenic peptide<sup>[58]</sup>. Results of the present study demonstrated that serum total ghrelin levels were insignificantly higher in the both stunted groups compared to the control group. Such observation agreed with previous studies which demonstrated insignificant difference in serum total ghrelin levels of Turkish children with

CDGP<sup>[59]</sup>, in short stature children in USA<sup>[60]</sup>, as well as in Iranian children with ISS<sup>[61]</sup> compared to control group. Our results are also in harmony with earlier reports in which significantly higher ghrelin levels were reported in the serum of Turkish short stature children with CDGP<sup>[54]</sup>, and malnourished Turkish children<sup>[62]</sup>, as well as in Polish short thin children<sup>[45]</sup> compared to control group.

It has been suggested that ghrelin levels might be elevated in Turkish short stature children with CDGP in a compensatory manner due to its orexigenic and strong GH secretagogue functions as a response to lowered body weight and height SDS<sup>[54]</sup>. Actually, ghrelin can act both directly on the pituitary to stimulate GH release by binding to GHSR1a and by acting on hypothalamus and vagus nerve<sup>[63]</sup>. Moreover, ghrelin analogs have been demonstrated to possess a potential beneficial effects in the treatment of GH-deficiency disorders<sup>[64]</sup>.

Our data also indicated that no correlation was observed between total ghrelin levels and IGF-1 or anthropometric measurements. These findings are in accordance with the study of *Pinsker et al.*<sup>[60]</sup> who reported that height, weight, and BMI z-scores did not correlate with ghrelin levels in short stature children in USA. Similarly, there was no significant correlation between ghrelin, growth hormone, IGF-1 levels, bone age and BMI in Iranian children with ISS<sup>[61]</sup>.

In the present study, serum levels of glucose, insulin, Beta cell function, and insulin resistance of all, normal and underweight, stunted children did not differ significantly from the control ones, suggesting lack of simple relationship between stunting and predisposition to diabetes among studied stunted Egyptian children. These results are in harmony with the findings of *Mamabolo et al.*<sup>[22]</sup> in which no significant difference was found in serum levels of glucose, insulin, Beta cell function, and insulin resistance in stunted South African children. On the same line, short German children exhibited insignificant changes in glucose, insulin, HOMA-IR compared to normal height children<sup>[23]</sup>.

On the contrary, decreased values of serum insulin and Beta cell function has been demonstrated in stunted Brazilian children<sup>[65]</sup>. Additionally, a recent study showed the presence of insulin resistance among Indian undernourished rural population that was improved after six months of food supplementation<sup>[66]</sup>. On the other hand, *Clemente et al.*<sup>[67]</sup> observed higher serum insulin levels in stunted Brazilian children. These inconsistent findings could be due to different degree of malnutrition, ethnicity, age ranges of participants, pubertal stage, body weight status, as well as methods of biochemical analyses.

In the current investigation, serum Ca and Mg levels were significantly lower in both stunted groups compared to control group. These observations are in accordance with the those of *Khairy et al.*<sup>[68]</sup> in which nutritionally stunted Egyptian children exhibited significantly lower

serum Ca and Mg levels compared to control group. Likewise, significantly lower serum Mg<sup>[69]</sup> and serum Ca<sup>[70]</sup> levels were demonstrated in nutritionally stunted Egyptian children and in stunted Chinese children, respectively. Actually, it has been reported that stunting and associated micronutrient deficiencies can cause permanent loss of growth and can lead to long term deficits in mental capacity in Pakistani malnourished stunted children<sup>[71]</sup>. Additionally, dietary Ca deficiency results in reduced serum Ca and increased parathyroid hormone (PTH) levels which increase bone resorption and osteoclast activity leading to bone loss<sup>[72]</sup>.

On the other hand, an important role for Mg in vitamin D activation and function as well as in bone metabolism has been reported. Thus, Mg deficiency was found to be associated with various disorders, such as skeletal deformities and osteoporosis<sup>[73,74]</sup>. Moreover, Mg deficiency may give rise to alterations in glucose metabolism, since Mg plays an important role in carbohydrate metabolism and insulin response, as a cofactor involved in transmembrane transport of glucose and release of insulin<sup>[75,76]</sup>. Studies have found that Mg deficiency is usually associated with endocrine and metabolic disorders, especially type 2 diabetes mellitus<sup>[77]</sup>. Indeed, this was evidenced in the current study by a significant negative correlation between Mg and glucose levels. Our finding is in accordance with the study of *Bo et al.*<sup>[78]</sup> who found significant negative correlation between dietary Mg intake and serum glucose levels in Italian pre-school children born with very low birth weight. It is also in harmony with the study of *Shahbah et al.*<sup>[79]</sup> in which Egyptian children with type-1 diabetes showed significant negative correlation between Mg and glucose .

In current study, the observation that stunted underweight children exhibited significantly lower serum Zn level agreed with the reports of *Aly et al.*<sup>[80]</sup> in which nutritionally stunted Egyptian children showed significantly lower serum Zn levels , and that of *Marasinghe et al.*<sup>[81]</sup> in severely stunted and severely underweight Sri Lankan children compared to control group. In fact, the role of Zn in growth had been explained through its influence on the GH/IGF-1 system, thus *Hamza et al.*<sup>[42]</sup> reported that Egyptian children with short stature and Zn deficiency demonstrated significant increases in serum IGF-1, and height SDS after 3 months of Zn supplementation. The authors suggested that the growth stimulating effect of Zn might be mediated through changes in circulating IGF-1 levels.

In conclusion, our findings highlight the importance of early detection of abnormalities in GH /IGF-1 axis and micronutrients levels in hope that appropriate intervention strategies could improve their status to obtain full growth potential in nutritionally stunted Egyptian children.

#### CONFLICT OF INTEREST

There are no conflicts of interest.

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