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Original article

Serum concentration and mRNA expression of Transforming Growth Factor-Beta 1 (TGF-β1) in stunted and non- stunted toddlers

Lina Nurbaiti [a, b, *], Nurpudji Astuti Taslim [c], Agussalim Bukhari [c], Mochammad Hatta [d]

[a] Doctorate Program, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[b] Department of Public Health, Faculty of Medicine, Mataram University, Mataram, Indonesia
[c] Department of Nutritional Sciences, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[d] Department of Molecular Biology and Immunology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia

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SUMMARY

Background and aims: West Nusa Tenggara Province has the fifth-highest prevalence of stunting cases in Indonesia. So far, limited research is available to understand the likelihood of stunting in this region. Transforming Growth Factor-Beta 1 (TGF-β1), an immunoregulatory cytokine, may affect the stunting progression. Knowledge of messenger mRNA expression in the TGF-β1 gene in stunted toddlers could help to determine therapeutic targets to catch up on their growth.

Objective: This study compared the expression of TGF- β1 mRNA and TGF-β1 concentrations in the stunted and the non-stunted toddlers. The nutritional status of all participants was also gathered and linked to the stunting issue.

Methods: A cross-sectional study was conducted on 48 toddlers aged 12–36 months. The stunting case was defined as a Z-Score of less than −2 of length/height for age according to WHO. The serum TGF-β1 and TGF-β1 gene mRNA were measured using ELISA and RT-PCR, respectively. The nutritional status data were collected through interviewer-administered structured questionnaire to the toddlers' parents and 48-h food recalls. Descriptive analyses were applied to determine the distribution of participants' macronutrient and micronutrient levels.

Results: Results show that there were significant differences in expressions of the TGF- β1 gene mRNA of the stunted and the non-stunted toddlers. The expression of the TGF- β1 mRNA gene in the non-stunted toddlers was also higher with 13.7 ± 0.859 fold change than those of the stunted toddlers with 9.01 ± 1.76 fold change with a p-value <0.001. The serum TGF-β1 concentrations in the stunted toddler (6.20 ± 3.60 pg/ml) were significantly lower than the ones in the non-stunted toddlers (14.3 ± 1.05 pg/ml) with p-value <0.001. However, there was no clear relationship between the likelihood of stunting and the nutritional status from the obtained data.

Conclusion: Overall findings demonstrate the significantly lower both the TGF-β1 gene mRNA expression and serum TGF-β1 for the stunted toddlers than the non-stunted toddlers, impacting bone formation and resorption. The outcomes of this study encourage the development of interventional therapy for stunted toddlers by increasing the serum TGF-β1 concentrations.

1. Introduction

Stunting is a short stature situation assessed by measuring body length or height. It is caused by inadequate nutrition or poor health conditions. Apart from directly affecting height, stunting has long-term impacts on intelligence, immunity, and productivity [1]. Indonesia has the fifth-highest rate of stunting in the world. The prevalence of stunting for Indonesian children under five is higher compared to neighboring countries, such as Vietnam (23%), Malaysia (17%), Thailand (16%), and Singapore (4%) [2]. The 2014 Global Nutrition Report showed that Indonesia has been one of the countries still facing the three nutritional problems, i.e., stunting, wasting, and/or overweight in children under five [3].

* Corresponding author. Doctorate Program, Faculty of Medicine, Hasanuddin University, Makassar. Indonesia.
E-mail address: dr.linanurbaiti@gmail.com (L. Nurbaiti).

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Based on the national basic health research, the proportions of short toddlers in Indonesia were 36.8% in 2007, 37.2% in 2013, and 30.8% in 2008, corresponding to around 7 million toddlers. West and East Nusa Tenggara are among the five provinces with the highest stunting rates (32%). Without any intervention, the stunting rate will threaten the future since it affects the quality of Human Resources (HR). West Nusa Tenggara province was ranked 30th out of 34 in Indonesia in terms of the Human Development Index, reflecting its poor human development performance [3].

Stunting associates well with bone physical growth and structure. Transforming Growth Factor-Beta 1 (TGF-β1) has been well known for its essential role in bone formation, mineral storage, and hematopoietic cell formation. Recent research shows the active role of TGF-β1 in bone metabolism and osteoimmunology [4]. Bone is a rigid organ with many functions, such as mechanical support for joints and tendons, protecting soft tissue or various organs from mechanical stress or trauma, mineral storage, hematopoietic cell formation, and hormone production.

The growth hormone is one of the factors affecting stunting [5]. The growth hormone regulates osteoblasts and osteoclasts during the growth of bones. Osteoblasts trigger the bone growth process, while osteoclasts inhibit the growth by providing some sort of control. TGF-β1 is an immunoregulatory cytokine that regulates immune cell proliferation, apoptosis, differentiation, and migration [6]. Besides, TGF-β1 plays essential roles in bone formation, mineral storage, and hematopoietic cell formation [7]. TGF-β1, which can be found in the bone matrix, also helps form and regulate osteoblasts and is active during bone formation [8,9].

Several micronutrients are required for adequate children's growth. Yet, it has been unclear which nutrient deficiency likely contributes to stunting in populations with the risk of poor nutrition. Several micronutrients, including zinc, iron, and vitamin A, have been associated with immune function and risk of morbidity, affecting growth [10].

By considering the important roles of TGF-β1 in bone formation, this study examines the correlation between TGF-β1 and stunting, which is scarcely available in the literature and is linked with the level of macronutrients micronutrients intake. This study combines molecular biology in assessing stunting cases, linked with the nutritional status of the participants. A cross-sectional study was conducted on 48 toddlers aged 12–36 months by measuring their serum TGF-β1 and TGF-β1 gene mRNA using ELISA and RT-PCR examination, respectively.

2. Materials and methods

The study populations were stunted and non-stunted toddlers aged 12–36 months in the Teruwai Community Health Centre, Central Lombok Regency, West Nusa Tenggara, Indonesia. All of them lived with biological parents and had a complete history of basic immunization. The stunting status was defined as a Z-Score < −2 of length/height according to the calculation by WHO. A total of 24 healthy toddlers in the stunted and the non-stunted group were selected. The required number of participants was determined statistically. The number of test participants for each group were minimized to reduce the costs. They were estimated using Cochran's method [11] by setting the confidence interval of 95% and a margin of error of 10%. The study participants were randomly selected from the total number of toddlers population of 1214, of which 75 suffered stunting resulting in the minimum required number of test participants of >23. Toddlers under the following conditions were excluded from the study: sick or had experienced sickness within the last three months, had congenital disabilities, a history of low body birth weight or premature birth, a history of labor by vacuum extraction or chunam, and had a history of asphyxia. The exclusion of those toddlers was done to exclude stunted toddler originated from infection, sickness, and/or congenital disorder.

The study was conducted via a cross-sectional mRNA expression of the TGF-β1 gene and serum TGF-β1 concentration in the stunted and the non-stunted toddlers. The TGF-β1 gene mRNA expressions were examined using RT-PCR, while the soluble serum TGF-β1 concentrations were examined using the ELISA method.

The collection and treatment of blood samples were done by following a method detailed elsewhere [12,13]. A blood sample of 100 μl taken from the veins was introduced into a tube containing 900 μl of L6 lysis buffer solution. The mixture was then centrifuged under a speed of 1200 rotations per minute (RPM) for 10 min. The supernatant was removed and the precipitate containing the extracted RNA was further centrifuged for 3 min under a speed of 1200 RPM, followed by the addition of 20 μl diatom suspension. The extracted RNA could then be settled down. Subsequently, the diatom suspension was constantly stirred by placing on a gyratory shaker set at a speed of 100 RPM for 10 min. The mixture of diatom suspension and the L6 lysis buffer was centrifuged again using an Eppendorf microcentrifuge under a speed of 12,000 RPM for 15 s. The supernatant was separated by careful sucking with a Pasteur pipet linked with a vacuum pump to leave around 10 μl of the residual suspension. The supernatant was then washed twice using 1 ml of washing L2 buffer. The L2 buffer of 1 ml was first added before the mixture was rotated and centrifuged (under a speed of 12,000 RPM for 15 s), followed by the removal of the supernatant. Then, the precipitate was washed again using 1 ml ethanol (70%) twice, followed by centrifugation for 15 s at a speed of 12,000 RPM. Afterward, the precipitate was washed by mixing with 1 ml of acetone, followed by centrifugation for 15 s at a speed of 12,000 RPM. The precipitate was then dried and the cap was let open in an oven at 50–55 °C for 10 min. The dry precipitate was then solubilized in a tube using a 60 μl of TE buffer, and stirred until well mixed. The tube was then incubated in an oven at 56 °C for 10 min. Subsequently, the mixture was centrifuged at a speed of 12,000RPM for 30 s. Finally, about 40–50 μl of the supernatant was then taken and stored at a temperature of −80 °C.

Measurements of the serum TGF-β1 concentrations were conducted using the ELISA (enzyme-linked immunosorbent assay) method detailed elsewhere [14], which were done in duplicate to ensure reproducibility. The TGF-β1 (LSBio®) analysis was done using the ELISA kit and ELISA Reader 270 (Biomerieux, France) with a wavelength of 450 nm within 30 min. The serum was taken from the storage (−80 °C) and stored in a freezer. Firstly, 100 μl of an assay diluent containing buffer protein was inserted into each well. Next, 100 μl of the standard solution was added. The standard solution contained a target recombinant mice protein TGF-β1 kit (LSBio, USA) which did not require inter- and intra-assay coefficient calculations. The mixture in the wells was then incubated for 2 h at room temperature. Afterward, the solution was sucked and washed for four times with phosphate-buffered saline (PBS), followed by the addition of 200 μl of the conjugate solution containing streptavidin HRP into each well and further incubated for 2 h at room temperature. Later, the solution was sucked and rewashed four times using PBS and added with 200 μl of TMB ELISA substrate solution into each well, before further being analyzed using the ELISA reader 270 (Biomerieux, French).

The measurements of the TGF-β1 mRNA expression were conducted using the RT-PCR examination (Real-Time System PCR Bio-Rad®) at The Molecular Biology and Immunology Laboratory, Faculty of Medicine, Hasanuddin University. The primers used were TGF-β1 were 5′-TGG CGA TAC CTC AGC AAC C-3′ and TGF-β1 ref of 5′-CTC GTG GAT CCA CTT CCA G-3′ genes. The housekeeping gene GAPDH was used as internal control GAPDH for the 5′-CCAGCCGAGCCACATCGCTC-

3′ gene and GAPDH Rev of 5′-ATGAGCCCCAGCCTTCTCCAT-3'. The RT-PCR was run under the condition of 95 °C for 10 s and 57 °C for 15 s performed under 40 cycles, adjusted according to the protocol suggested earlier [15], where the RT-PCR employed the SYB Green dye-based RT-PCR master mix kit in one stage. This protocol was optimized in the RT-PCR machine CFX Connect System (USA) instrument by adjusting the color according to the guideline.

To allow linkage between the expression of TGF- β1 mRNA and TGF-β1 concentrations and the nutritional status of the participants, an interviewer-administered structured questionnaire, and 48-h food recall were used to collect the information on the nutritional data. For the children, the consent for participation in the study was signed by their parents; and the questionnaire-based interview was conducted by their parents. The nutrient intake data were processed using Nutrisurvey 2007 software to obtain the level of energy consumption, the level of macronutrients, and micronutrient intake.

The data obtained were processed and statistically analyzed using the Chi-square, the Independent t-test, and the Pearson correlation test to evaluate both the TGF-β1 protein concentrations and TGF-β1 mRNA expression. Descriptive analyses were applied to determine the levels of macronutrients and micronutrients distributions of the participants.

Apart from identifying the role of the TGF-β1 on stunting, several stunting risk factors were addressed in the participants to justify the findings. The risk factors included feeding frequency, energy consumption rate, level of macronutrient consumption (carbohydrate, protein, fat), and level of micronutrient (Vitamin A, Calcium, iron, and Zinc) consumption.

3. Results

3.1. TGF-β1 in stunted toddler

3.1.1. Participant's anthropometric characteristics

Table 1 compares anthropometric characteristics of the stunted and non-stunted toddlers. The physical indicators of stunted participants can be seen clearly from their weight and height. Despite having similar average age, all stunted toddlers had significantly lower weight (9.99 ± 0.74 vs 12.8 ± 2.5 kg) and lower height (81.1 ± 3.23 vs 88.5 ± 4.85 cm). The Z-scores of the stunted groups in terms of weight and height were 0.59 and 0.69 below the means, respectively. It means that the differences in weight and height between the stunted and the non-stunted groups were higher than 1.18 and 1.38 times the respective standard deviation. Moreover, the t-test results show that both the weight and the height of the stunted group were significantly lower than the whole participants with p-values of 0.0065 and 0.0035, respectively.

3.1.2. Gender proportion

Table 2 shows the gender proportion of the two participant groups. Based on the Chi−Square Test, there was a significant relationship between gender with nutritional status ($p < 0.05$). The number of girls who suffered from stunting was higher (72%) than boys (26.1%). This finding represents the situation in the sampling location. It does not universally apply. However, the finding may imply that being a female is a risk factor influencing stunting. Such deduction, however, shall be further analyzed in detail in the future study. Evidence from earlier studies shows that being a female has been reported as one of the risk factors for stunting in Northern Ethiopia [16].

Table 1
Nutritional status of the participants.

Variable	Nutritional Status				
	Stunting		Non-stunting		P-value
	Mean	SD	Mean	SD	
Age (month)	30.5	3.87	30.1	4.2	0.502
Z-Score H/A	−2.96	0.58	−0.81	0.82	0.058
Z-Score W/A	−2.18	0.53	−0.41	0.85	0.020

Table 2
Gender proportion of the participants.

Gender	Nutritional Status				
	Stunting		Non-stunting		P-value
	n (24)	%	n (24)	%	
Boy	6	26.1	17	73.9	0.004
Girl	18	72	7	28	

3.1.3. Concentration of TGF- β1 serum

Figure 1A shows that there is a clear difference between the concentration of TGF-β1 serum for the stunted group (6.20 ± 3.60 pg/ml) and the non-stunted group (14.3 ± 1.05 pg/ml). Based on the independent sample t-test between the nutritional status of the stunted and the non-stunted toddlers, TGF-β1 serum concentrations were found to be significantly lower for stunted than the non-stunted toddlers with $p < 0.001$.

3.1.4. mRNA expression

Figure 1B shows the analysis with an independent sample t-test for mRNA expression between groups of the stunted and non-stunted toddlers. Results show that the mRNA expression of the TGF-β1 gene in non-stunted toddlers was higher (13.7 ± 0.859-fold change) than in the stunted ones (9.01 ± 1.76 fold change) with a p-value < 0.001. The Pearson correlation test between the mRNA Expression of the TGF-β1 gene and TGF-β1 serum concentrations against stunted and non-stunted toddlers found a strong correlation with a p-value of <0.001 and correlation coefficient of $r = 0.935$.

3.2. Other risk factor analysis

To assess risk factors of stunting and confirm their effect on the participants in this study, a few factors/parameters were evaluated, namely: feeding frequency, energy consumption level, the consumption level of macronutrients, and lastly, the consumption level of micronutrients.

3.2.1. Feeding frequency

The daily feeding frequency for the participants was classified into two categories, namely the frequency of eating <3 times a day and ≥3 times a day. The distribution of daily feeding frequency for the participants can be seen in Fig. 2. In the stunted toddler group, most of the participants had a frequency of eating <3 times a day (70.8%). Meanwhile, the participants of the non-stunted toddlers mostly have a frequency of eating >3 times a day (75.0%). It was observed that for the community where the study was conducted, the frequency, and texture of the food were appropriate, but the amount, variety, and hygiene were still inaccurate. As such the feeding practice is one of the specific nutritional interventions that can be carried out to prevent and overcome stunting [17,18].

3.2.2. Energy consumption rate

The levels of energy consumption in Fig. 3 were categorized into four based on recommended dietary allowance (RDA): severe deficit (<70%), mild deficit (70−80%), normal (81−120%), excess

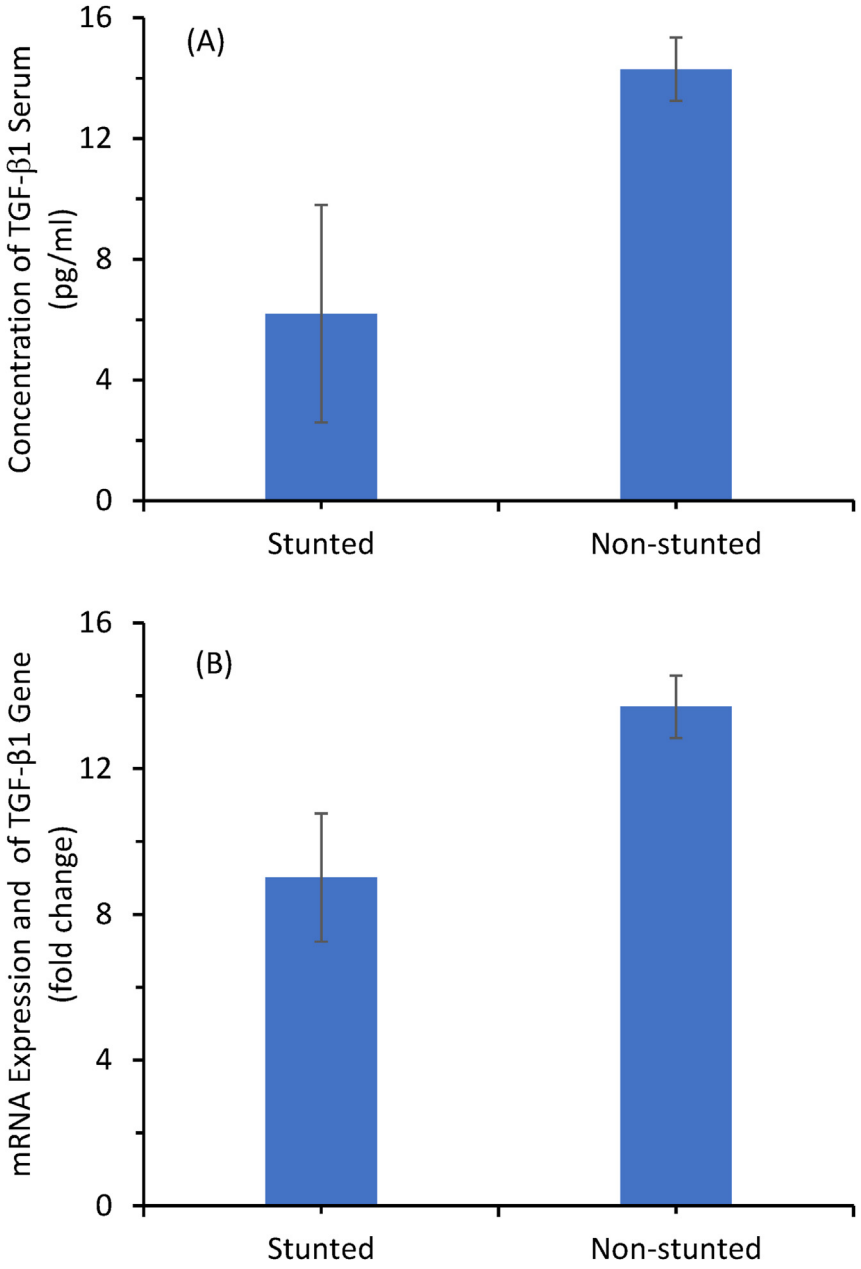

Fig. 1. (A) Concentrations of TGF- β1 serum of the stunted (n = 24) and the non-stunted (n = 24) participants (P < 0.001), and (B) the mRNA expression of TGF- β 1 gene of the stunted and non-stunted participants (P < 0.001).

(>120%). Based on the categories, the average energy consumption of the participants was 804.1 kcal, with the lowest energy consumption of 269.1 kcal and the highest of 1654.2 kcal.

3.2.3. Level of macronutrients' consumption

The macronutrient uptakes for the participant toddlers including carbohydrate, protein, and fat consumption are summarized in Fig. 4. The average protein consumption for the toddlers was 30.1 g (lowest-highest: 4.5–77.0 g), fat 32.8 g (lowest-highest: 2.9–73.7 g), carbohydrates 95.3 g (lowest-highest: 14.5–192.8 g). Based on Fig. 4, most of the levels of carbohydrate consumption fell under the severe deficit category, in which the stunted group was 79.1% and the non-stunted toddler group was 70.8%. Most of the protein consumption levels in the stunted group were in the normal category (41.7%). Meanwhile, in the non-stunted group, the level of protein consumption was in the excess category (50.0%). Most of the levels of fat consumption in the stunted group fell under the severe deficit category (54.2%). Meanwhile, in the non-stunted group, the levels of fat consumption were mostly in the normal category (41.7%).

3.2.4. Level of micronutrients consumption

The levels of micronutrient consumption consist of vitamin A, calcium, iron, and zinc. The distribution of micronutrient consumption levels for the participants in this study is presented in Fig. 5. The average consumption of vitamin A was 228.9 μg (ranged from 1.5 to 10.345.0 μg), calcium 129.7 mg (ranged from 11.3 to 1575.0 mg), Fe 3.66 mg (ranged from 0.8 to 17.5 mg), and zinc 3.3 mg (ranged from 0.7 to 9.1 mg). Most of the levels of consumption of vitamin A, calcium, iron, and zinc in the stunted and non-stunted children were in the severe deficit category. The levels

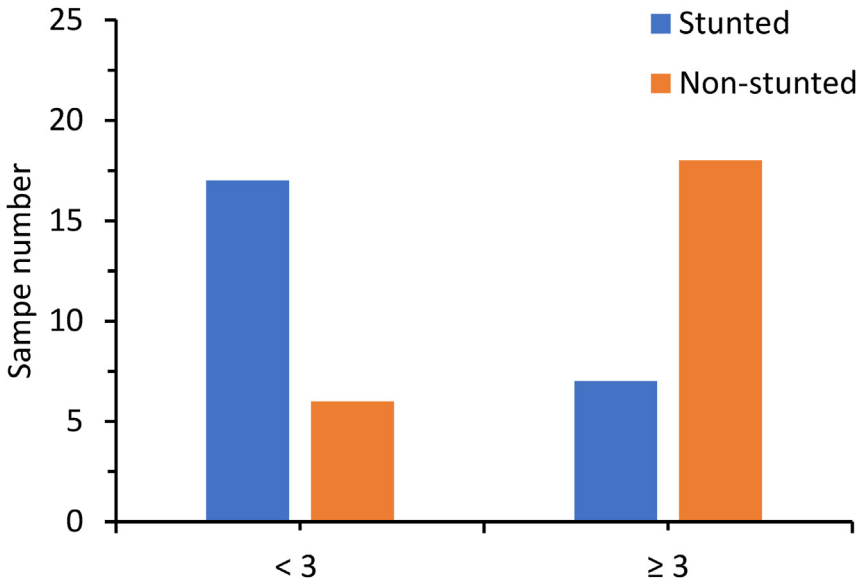

Fig. 2. Daily meal frequency of the participants.

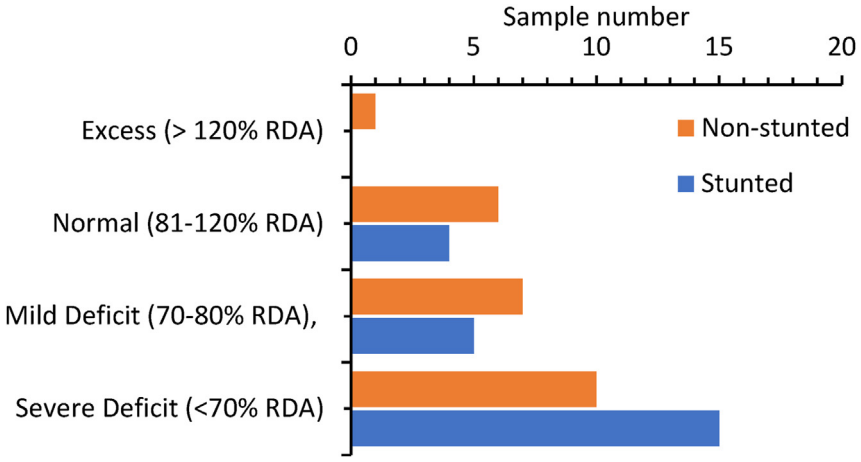

Fig. 3. Distribution of participants' energy consumption levels, categorized based on the 2012 energy adequacy of 1125 kcal of recommended dietary allowance.

of vitamin A, calcium, iron and zinc consumptions for the non-stunted and stunted groups were 83.3% and 79.2%, 95.8% and 83.3%, 79.2% and 70.8%, and 91.6% and 83.3%, respectively.

4. Discussion

This study demonstrates significant differences in the expression of the TGF-β1 gene mRNA and serum TGF-β1 concentrations between the stunted and the non-stunted toddlers. The TGF-β1 gene mRNA has a strong association between bone cells [19] and eventually can be associated with the likelihood of stunting. TGF-β1 has an important role in bone formation, mineral storage, and hematopoietic cell formation [20,21]. TGF-β1 interacts with bone component cells, such as osteoblasts, osteoclasts, chondrocytes, mesenchymal stem cells, hematopoietic rods, and other hormones [4,22].

The results of this study are in line with the previous study, which states that bone resorption can be induced by increased T-cell activation and TNF-alpha production and that TGF-β1 signaling prevents bone loss [7]. There are indirect roles of TGF-β1 in affecting stunting through its roles in bones developments. Other reports also mentioned that factors such as TGF-β1 and Bone Morphogenic Proteins (BMP) affect bone cell function. The resorption and bone formation systems allow bone formation waves to follow each cycle of bone resorption [8,9]. TGF-β1 is found in bones and serves to inhibit osteoblast differentiation. TGF- β1 inhibits the CBFA1 expression and the osteocalcin gene mediated by Smad3. Smad3 repression mediated by CBFA1 functioned in the regulatory mechanism of osteoblast differentiation inhibition by TGF-β1 [23,24].

Another TGF-β1 family that has an important effect is BMP. BMP begins the cartilage formation and the osteogenesis [8]. Both TGF-β1 and BMP involve in most cellular processes. The coordinated activity of Smads activated by Runx2 and TGF-β or BMP is important for skeletal formation. TGF-β1 inhibits the proliferation of myoblasts and the formation of fibrosis. Specific inhibition by TGF-β1 can increase the skeletal muscle's ability to regenerate [25–27].

Compelling evidence from earlier work has demonstrated a strong association between the immune and skeletal systems (so-called Osteoimmunology), including the critical role of TGF-β1 in the development and maintenance of the skeletal tissue [7], as well as stunting, as demonstrated in this study. Bone remodeling is an

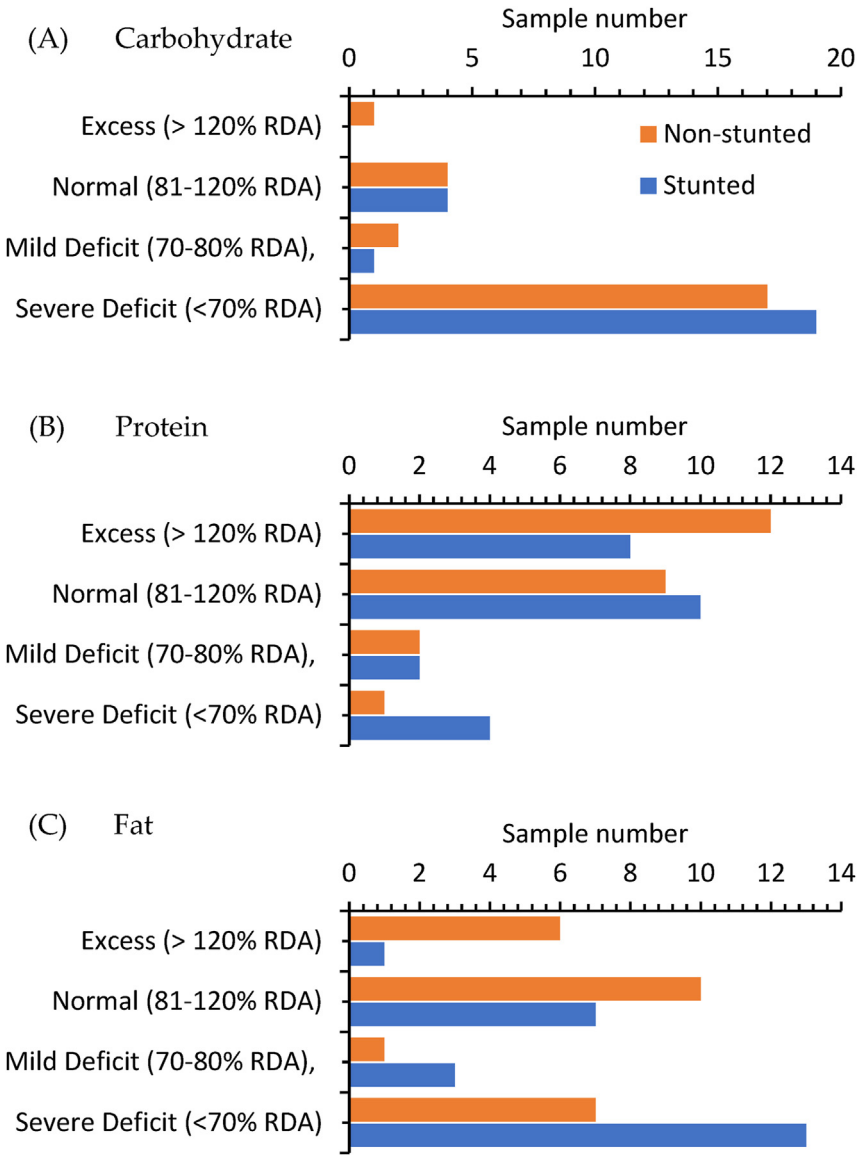

Fig. 4. Distribution of the participants macronutrient consumption levels.

active and dynamic process that depends on the correct balance between bone resorption by osteoclasts and osteoblasts' bone deposition [7]. The bone remodeling process consists of three phases: bone resorption initiation by osteoclasts, the transition from resorption to new bone formation, and bone formation by osteoblasts. Those processes occur due to the osteoclasts, osteoblasts, osteocytes, and bone layer cell coordination, which form a temporary anatomical structure called the Basic Multicellular Unit [4].

As the matrix grows, osteoblasts and osteocytes communicate with each other. Osteoblasts secrete organic matrices that are solid collagen [28]. Osteoclast precursors originate from the spleen and liver and then travel to blood vessels close to the newly formed bone trabeculae. Osteoclast precursors combine to form multinucleated osteoclasts and absorb most of the newly formed bone. Osteoblasts and osteoclasts must be balanced to maintain skeletal integrity and calcium metabolism [29].

A very strong correlation between TGF-β1 serum concentrations with the mRNA expression of the TGF-β1 gene is shown from the Pearson correlation coefficient. It implies that for stunted toddlers, both the TGF-β1 serum concentrations and the mRNA expression of the TGF-β1 gene are significantly lower than that of the non-stunted toddlers. Such finding can be explained as follow. Stunting is preceded by intestinal mucosal damage and microbiome changes associated with systemic inflammation [30]. Indeed, there is an established association between stunting and environmental enteric disorder. A small-intestine enteropathy is highly prevalent in low-resource countries and is characterized by mucosal inflammation, small intestine villi flattening, and increased intestinal permeability [31]. These disorder's triggers are probably inadequate diet, continuous exposure to environmental contaminants, and/or an alteration of the normal commensal flora [30]. In the gut, intestinal epithelial cells (IECs) as well as selective immune cells, including dendritic cells (DCs), are important sources of bioactive TGF-β [32]. Epithelial cell injury and gut inflammation have both been demonstrated to enhance TGF-β production by IECs [33]. There is also growing evidence to suggest that the microbiota also regulates IEC-derived TGF-β. Several Clostridium species have been shown to produce short-chain fatty acids, such as butyrate, acetate, and propionate, capable of exacerbating TGF-β production by colonic Ecs [34]. Alcaligenes species, gut-associated lymphoid

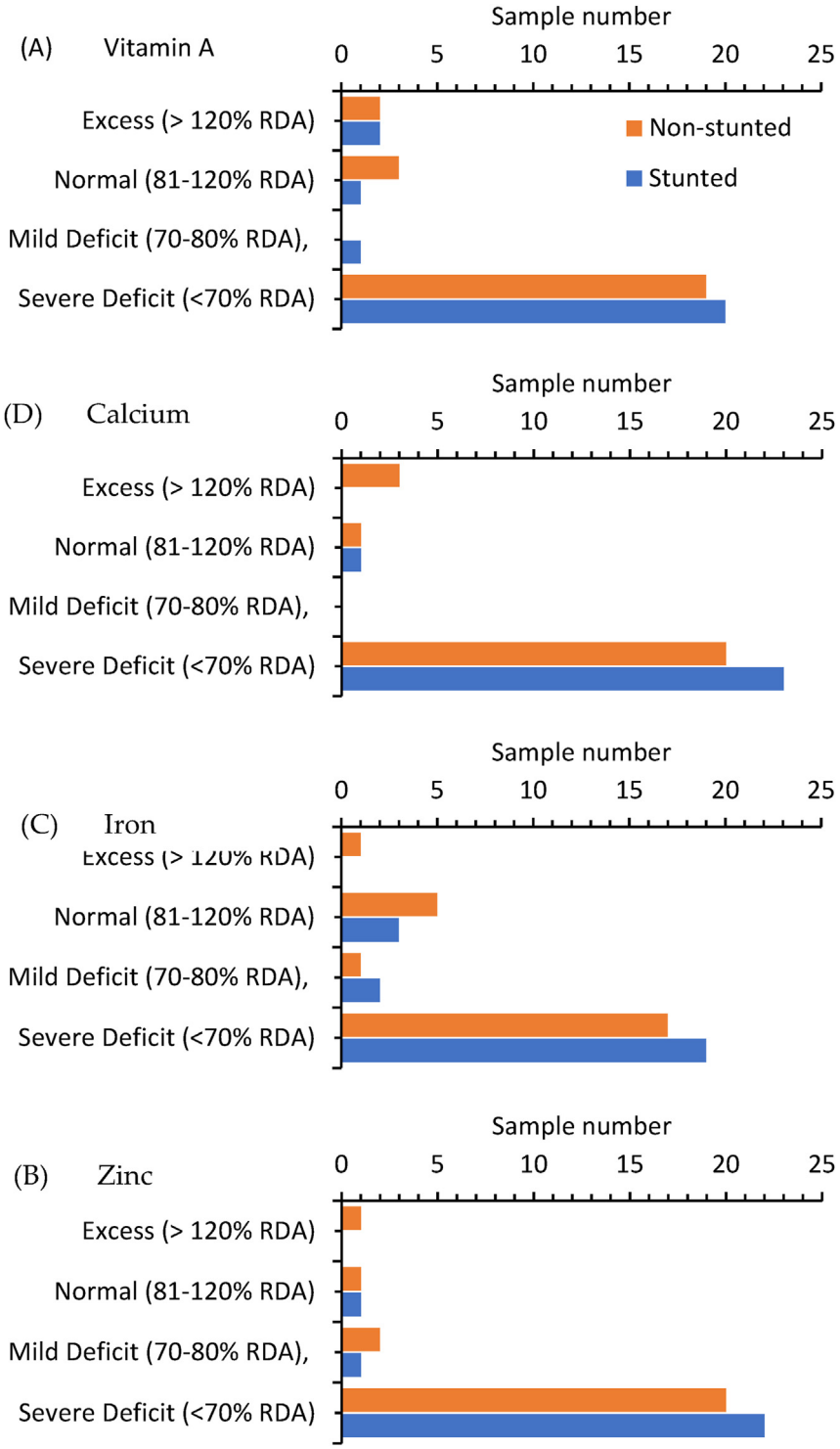

Fig. 5. Micronutrient consumption levels (Vitamin A, Calcium, Iron, and Zinc) based on recommended dietary allowance for vitamin A of 350 μg, calcium of 500 mg, Fe of 8 mg and zinc of 7 mg.

tissue-resident bacteria, as well as *Lactobacillus gasseri* SBT2055, a probiotic bacterium, have been shown to induce TGF-β production by small intestinal DCs in a TLR2-dependent manner, then induce IgA production by B cells [35]. Thus, by influencing TGF-β production in the gut, the microbiota regulates IgA secretion to strengthen the intestinal barrier [36].

The results show a strong relationship between energy intake and the number of daily meal intake. The stunted participants mostly had <3 daily meal intake per day and they were under high deficit in terms of energy consumption level (Fig. 3). It follows that appropriate energy intake is highly important for toddlers for growth purposes.

Incorrect consumption behavior causes low food consumption and affects children's nutritional status [37], which is partly also demonstrated by the results in this study (Figs. 2–5). The protein consumption levels in Fig. 4C show that the level of protein adequacy was mostly normal and/or in excess for the stunted group. It follows that there was no clear relationship between protein deficiency and stunting. This finding contradicts other studies [38,39] that state that there is a significant relationship between protein deficiency and the incidence of stunting in children under five. Stunting is caused by a chronic shortage of macro and micro substances from the womb to toddlers' age. Measurement of nutritional status using a 2-day food recall does not fully represent the whole nutritional status. Still, results on the food diversity score, meal frequency questionnaire, and qualitative research methods in this study can explain the phenomenon of nutritional deficiency, and in accord with another study [22]. Energy and protein are needed to form long bone growth plates [40]. Protein plays a role in carrying the growth hormone, or growth hormone to the growth plate and muscles so that the body gets longer. If protein and energy deficiency occurs chronically, there will be a disturbance in the growth hormone's balance [38,39].

The bones in humans begin to grow from the embryo, at the age of 6–7 weeks from the mother's womb's fertilization process. The growth continues until it is completely composed of 3 months of gestation. In this phase, the bone formation process is influenced by calcium and placental hormones [41]. The bones that are formed are still very soft. However, the bone will continue to grow and harden until labor arrives. After birth, the process of bone formation in infants will be influenced by calcium [42], their daily activities [43], and also influenced by growth hormones [44,45]. Growth hormone influences osteoblasts and osteoclasts for the process of bone growth. Osteoblasts work by triggering the bone growth process, while osteoclasts work by inhibiting this process. In this phase, the bones formed are cartilages (cartilages) whose texture is still very soft, and the color is still transparent [9,46].

Stunting can be caused by chronic macronutrient and micronutrient deficiencies from the womb to the age of toddlers and beyond [47,48]. Measurement of nutritional status using 48 h food recall only describes the short-term nutritional status, so further research is needed using the food diversity score method, food frequency questionnaires, and qualitative research methods to explain the phenomenon of nutritional deficiency.

Further research is needed to explain the pathomechanism and role of TGF-β1 and other molecules in the process of stunting. Furthermore, research on the use of serum TGF-β1 concentrations as early detection of stunting in toddlers is warranted. These would provide systematic management to overcome stunting and define therapeutic targets for pursuing the growth of stunted toddlers. The development of interventional therapy for stunted toddlers can be considered by increasing the serum TGF-β1 concentrations with nutritional therapy and/or other therapies that are simple and cost-effective.

5. Conclusions

This study demonstrates a significant difference in mRNA expression of the TGF-β1 gene between the stunted and the non-stunted toddlers. The mRNA expression of the TGF-β1 gene in non-stunted toddlers was significantly higher than in stunted toddlers. Moreover, a significant difference was observed between the serum TGF-β1 concentrations of stunted and non-stunted toddlers, where the serum TGF-β1 concentrations in the non-stunted toddlers were significantly higher than in the stunted toddlers. There was no clear relationship between the likelihood of stunting and the nutritional status. Nonetheless, the stunted toddlers had significantly lower energy and daily meal intake. This research can be a basis to develop early detection and stunting intervention modalities in individuals and the community via TGF-β1 and mRNA expression detections.

Author contributions

Conceptualization, A.B and L.N; methodology, L.N.; validation, L.N.; formal analysis, L.N.; investigation, L.N.; resources, L.N.; data curation, L.N.; writing—original draft preparation, L.N.; writing—review and editing, N.A.T., A.B. and M.H.; visualization, L.N.; supervision, N.A.T., A.B. and M.H. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of Medical Faculty Hasanuddin University (protocol code1038/UN4.6.4.5.31/PP36/2019 approved in 2019). All experiments have been examined and approved by the appropriate ethics committee and have been performed under the ethical standards. All research participants have been given informed consent. For the children, the consent was signed by their parents.

Informed consent statement

Informed consent was obtained from all participants involved in the study.

Declaration of competing interest

The authors declare no conflict of interest.

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