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Original Article

Bone turnover markers and bone mineral density in prepubertal obese children

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Abstract

Background Growing evidence suggests that childhood obesity has an impact on bone metabolism. Its entails of bone resorption, destruction of mature mineralized bone by osteoclasts followed by ossification, bone formation by osteoblasts, to maintain the dynamic nature of bone. Serum C-telopeptide of collagen cross-links (CTX) is considered a bone resorption marker while serum procollagen type I N-propeptide (PINP) is considered abone formation marker. Previous studies have reported the abnormality of these bone turnover marker in obese children.

Objective To compare bone turnover markers and bone mineral density (BMD) in obese prepubertal children to those of normoweight children.

Methods Bone metabolism was evaluated by measuring serum PINP as a bone formation marker and CTX level as a bone resorption marker by enzyme-linked immunosorbent assay. We used dual-energy X-ray absorptiometry (DEXA) scan to evaluate BMD in 60 prepubertal children with obesity and 30 healthy prepubertal normoweight children.

Results The CTX was significantly higher in the case group compared to the control group (P=0.001). The case group also had significantly lower mean BMD (P=0.001) and BMD Z-score (P=0.001). C-telopeptide of collagen cross-links in the case group had significant positive correlations with waist circumference (P=0.001), BMI (P=0.001), and BMI Z-score (P=0.001). Significant negative correlations were found between waist circumference, BMI, and BMI Z-score with procollagen type I N-terminal propeptide, BMD, and BMD Z-score.

Conclusion Obesity has a negative impact on bone health. Low BMD was associated with high CTX in prepubertal obese children. **[Paediatr Indones. 2024;64:473-82; DOI: https://doi.org/10.14238/pi64.6.2024.473-82**].

Keywords: *procollagen type I N-terminal propeptide; C-terminal collagen type I extension propeptide; dual-energy radiograph absorptiometry; obesity; prepubertal*

Over the last three decades, the global public health burden of childhood obesity has increased. When overweight status continues into maturity, the chance of acquiring chronic illnesses at a young age rises dramatically.[1]

Bone structure and quality in young people with obesity result from the cumulative effects of immune-modulating and pro-inflammatory cytokine release as well as mechanical overload.[2,3] Waist circumference reflects visceral obesity, which is linked to lower bone mass density (BMD), implying that fat, notably visceral fat, is unfavorable to bone mass.[4]

For ossification, bone formation by osteoblasts, procollagen units are broken down into procollagen type I N-propeptide (PINP) and procollagen type-I C-terminal peptide (PICP) prior to type I collagen molecules being assembled into fibers.[5,6] serum contains two different forms of PINP: low-molecular-weight monomeric peptides and trimeric "intact"

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peptides.[6] Three factors explain why bone accounts for most of the serum PINP and PICP: 90% of proteins that make up bone structure are type I collagen; the skeleton is heavier than other tissues that contain type I collagen and has a faster metabolic rate. Therefore, there is a substantial correlation between bone formation indices and serum PINP and PICP levels.[7]

For bone resorption, destruction of mature mineralized bone in healthy children. The bone matrices that comprise a mature individual type I collagen's triple helices are broken down by osteoclast-derived tartrate-resistant acid phosphatase (TRAP) and cathepsin K, liberating fragments that include carboxy telopeptides (CTX) and amino telopeptides (NTX).[8] The CTX has grown in popularity as a biomarker for examining osteoclastic bone resorption activity because it can be quantified in serum samples using automated chemistry analyzers.[9,10]

Dual-energy radiograph absorptiometry (DEXA) scan can be used to evaluate the pediatric bone mineral density (BMD). In young people, the lower spine (L1-4) and the entire body, excluding the head, are the preferred skeletal sites for DEXA measurements.[11,12] According to guidelines, children's BMD needs to be corrected for height or age, or it can be juxtaposed to reference data using age-, gender, and height-specific Z-scores. In children, BMD Z-score that is < 2.0 standard deviation is considered low for age.[11,12]

Growing evidence implies that pediatric obesity influences the condition of bones. Thus, the study's goals were to compare CTX and PINP as bone turnover markers and BMD in obese children to those of normoweight children to assess the impact of obesity on bone metabolism in prepubertal children. We also analyzed for correlation between CTX, PINP, BMD, with body mass index (BMI) and waist circumference (WC) in obese children.

Methods

A case-control study was conducted in the Pediatric Tanta University Hospital from October 2022 to May 2023. In the case group, prepubertal obese children aged 5-10 years with BMI <95th percentile for minimum 6 months, and on an unrestricted diet were included. The control group was similar composition, normoweight children with BMI 5th-85th percentile. Egyptian growth curves were used for height, weight, and BMI calculation.[13] Exclusion criteria include hormonal disorders, inheritable conditions, chronic illnesses that might impair their growth, development, and pubertal maturation, on medications that impact bone health (calcium supplementation, vitamin D, antiepileptics, bisphosphonates, or steroid compounds), bone fractures or other bone diseases, acute illnesses causing inflammatory changes, and/or pubertal development.

All subjects underwent complete history taking and medical checkups including anthropometric measurements, using standard equipment in accordance with the International Biological Program's recommendations.[14] The mean of three subsequent readings was used for every measurement. A Seca scale was used to determine body weight while a Harpenden stadiometer was used to measure body height.[15]

Height (HT) Z-score and weight (WT) Z-score were calculated as the number of standard deviations of the actual height or weight of a child from the median height or weight of the children of his/her age as determined from the standard sample. This is prefixed by a positive sign (+) or a negative sign (-) depending on whether the child's actual height or weight are more than the median height or weight or less than the median height or weight. From -2 to +2 Z scores are classified as in range of median height or weight. More than +2 Z scores are classified as more than the median height or weight. Less than -2 Z scores are classified as less than the median height or weight.[16] Children were considered obese for BMI ≥95th percentile.[17] Waist circumference (WC) was measured in the midaxillary line halfway between the inferior margin of the ribs and the top of the hip bone.[18] Children were classified as prepubertal by direct examination using Tanner's staging criteria. One qualified investigator carried out Tanner's pubertal staging.[19,20]

Between 8:00 and 10:00 a.m., peripheral blood specimens were taken from the antecubital vein while subjects were prone and placed into one EDTA tube and two serum tubes containing a clot activator. The blood was then centrifuged at 3000 RPM for 15 minutes. Alkaline phosphatase, serum ionized Ca and inorganic phosphorus (Ph), parathyroid hormone (PTH), and 25(OH) vitamin D levels were all

measured on the same day as collection. The second serum sample was stored at -20°C until they were tested for PINP and CTX.

Diestro electrochemical detector with ISE calibrating package from *Diestro*, Argentina (catalog # IN0100) was used to determine serum ionized *Ca. A KONELAB PRIME 60i* was used to detect serum inorganic Ph and alkaline phosphatase (ALP) using reagents from *Thermo Fisher Scientific Oy-Finland* (catalog #: TR30026 and TR11320, respectively). Normal range of serum Ph in children was 4.5-6.5 mg/dL.[21] Vitamin D and PTH were measured using an automated chemistry analyzer (*Cobas 6000*) with *ROCHE Diagnostic* kits (REF: 07464215 190 and 11972103122, respectively). *Sun Red Human PINP* ELISA kits (catalog #201-12-1351) and *Sun Red Human CTX-I* ELISA kits (catalog #201-12-1350) were used to measure the concentrations of PINP and CTX, respectively.

Bone mineral density and BMD Z-score were measured in the lumbar spine using a DEXA scan on a *Lunar DPXIQ-USA* with pediatric software 4.5. Subjects' DEXA scans were performed on the same machine and analyzed by one radiology technician. Standard body positioning techniques were used to take the measurements.

Power analysis revealed that a sample size of 51 children in the case group and 25 participants in the control group were necessary to achieve a power of 80% with alpha=0.05. *SPSS* software was used to process data. Chi-square test was used to compare qualitative data and student t-test was used to compare quantitative parameters. Pearson's correlation test was used to assess the relationships between parameters. P values > 0.05 were considered to be statistically significant.

Results

The 60 prepubertal, consisted of 60 obese children in the case group and 30 normoweight children in the control group were followed this study. Obese subjects had a mean age of 7.11 (SD 1.38) years, and there were 31 females (51.7%) and 29 males (48.3%). The 30 normoweight subjects had a mean age of 7.18 (SD 1.41) years, and there were 14 males (46.7%) and 16 females (53.3%) There were no significant differences in serum ionized Ca, serum Ph, and PINP between the two groups (P=0.207, 0.542, and 0.312, respectively). While vitamin D was significantly higher in the control group than in the case group, PTH, ALP, and CTX were significantly higher in the case group than in the control group (P=0.001). In addition, the case group mean BMD and BMD Z-score were significantly lower than those of the control group (P= 0.001 for both) (**Table 1**).

Table 2 shows that there were no significant differences in PINP (P=0.259), CTX (P=0.348), BMD (P=0.361), BMD Z-score (P=0.381) between male and female case subjects.

The BMI, BMI Z-score, and WC showed significant positive correlations with CTX. In addition, BMI, BMI Z-score, and WC had strong negative correlations with BMD, and BMD Z-score in the case group (**Table 3** and **Figures 1, 2, and 3**).

In the case group, there were significant inverse associations between CTX and PINP, CTX and aBMD, and CTX and BMD Z-score. A strong positive correlation was found between PINP and BMD and PINP and BMD Z-score (Table 4).

Discussion

The effects of obesity on bone metabolism in prepubertal children were studied by comparing serum markers of bone turnover and BMD in obese and normoweight peers. Secondary objectives were to identify potential relationships between bone turnover indicators, BMD, BMI, and WC in obese children. In our investigation, the case group had significantly greater height, Ht Z-score, weight, Wt Z-score, BMI, and WC compared to the control group as expected.

We found no significant differences in serum ionized Ca or serum inorganic Ph levels between the case and control groups. However, the case group had significantly higher PTH and ALP levels than the control group. Also, vitamin D level was significantly lower in the case group than in the control group.

Our results agreed with other research,[22-26] which reported that 25(OH)vitamin D levels were low in obese subjects and were inversely related to BMI and body fat percentage. This could be the result of vitamin D is sequestered in fat, so that it would decrease the concentration of vitamin D in

Table 1. Analysis of subjects' demographic, bone markers, and BMD

Characteristics	Case (n=60)	Control (n=30)	P value
Age, years			0.820
Range	5-9.5	5-9.5	
Mean (SD)	7.11 (1.38)	7.18 (1.41)	
Sex, n(%)			0.881
Male	29 (48.3)	14 (46.7)	
Female	31 (51.7)	16 (53.3)	
Height, cm			0.001*
Range	106-143	105-129	
Mean (SD)	124.4 (8.97)	117.25 (6.51)	
HT Z-score			0.001*
Range	0.11-3.55	-1.22 - 1.55	
Mean (SD)	1.55 (0.82)	0.21 (0.76)	
Weight, kg			0.001*
Range	56.5-77	50-67	
Mean (SD)	65.13 (5.36)	57.30 (4.29)	
WT Z-score			0.001*
Range	2.1-3.99	-1.22 - 1.66	
Mean (SD)	2.84 (0.480	0.22 (0.82)	
BMI, kg/m^2			0.001*
Range	20-32	14.8-19.1	
Mean (SD)	24.83 (3.89)	16.32 (0.92)	
BMI Z-score			0.001*
Range	2.1-3.5	-1 - 1	
Mean (SD)	2.88 (0.44)	-0.06 (0.63)	
Waist circumference, cm			0.001*
Range	63-79	49.7-64.9	
Mean (SD)	70.60 (4.54)	57.07 (4.35)	
Vit D, ng/mL			0.001*
Range	4.3-34.6	13.36-49.14	
Mean (SD)	18.95 (7.23)	26.81 (11.14)	
Parathyroid, pg/mL			0.001*
Range	66-102	51-87	
Mean (SD)	82.29 (10.75)	69.73 (10.51)	
Alkaline phosphatase, IU/L			0.001*
Range	350-682	250-498	
Mean (SD)	521.88 (95.65)	330.43 (67.21)	
Serum ionized calcium, mmol/L			0.207
Range	1.2-1.32	1.2-1.32	
Mean (SD)	1.25 (0.03)	1.26 (0.04)	
Serum Ph, mg/dL			0.542
Range	4.5-6.5	4.5-6.5	
Mean (SD)	5.45 (0.58)	5.37 (0.55)	
PINP, ng/mL			0.312
Range	155.77-1,643.1	193.9-1,782.26	
Mean (SD)	581.73 (335.66)	662.70 (395.18)	
CTX ng/mL			0.001*
Range	20.2-96.07	4.44-17.49	
Mean (SD)	54.15 (23.37)	7.80 (3.67)	
BMD, g/m^2			0.001*
Range	-6.3 - -2.7	-1 – 1	
Mean (SD)	-4.30 (1.13)	0.02 (0.54)	
BMD Z-score			0.001*
Range	0.5-0.71	0.74-1	
Mean (SD)	0.61 (0.07)	0.87 (0.07)	

Table 2. Analysis of bone markers and BMD in the case group by sex

Variables	Male (n=29)	Female (n=31)	T-test	P value
PINP, ng/mL			1.141	0.259
Range	181.22-1,643.1	155.77-1,433.2		
Mean (SD)	632.71 (361.12)	534.03 (308.22)		
CTX, ng/mL			0.947	0.348
Range	20.2-88.16	21.46-96.07		
Mean (SD)	51.19 (22.96)	56.91 (23.78)		
BMD, g/m2			0.924	0.361
Range	-6 - -2.7	-6.3 - -2.7		
Mean (SD)	-4.16 (1.12)	-4.43 (1.15)		
BMD Z-score			0.883	0.381
Range	0.51-0.71	0.5-0.71		
Mean (SD)	0.62 (0.07)	0.60 (0.07)		

Table 3. Correlation coefficient analysis of BMI, BMI Z score, and WC with bone markers and BMD in the case group

Variables	BMI		BMI Z-score		WC	
	r	P value	r	P value	r	P value
CTX	0.547	0.001*	0.630	0.001*	0.561	0.001*
PINP	0.038	0.724	0.036	0.737	0.117	0.273
BMD	-0.774	0.001*	-0.858	0.001*	-0.745	0.001*
BMD Z-score	-0.798	0.001*	-0.876	0.001*	0.776	0.001*

Table 4. Correlation coefficient analysis of bone markers and BMD in the case group

Variables	PINP		CTX		BMD	
	r	P value	r	P value	r	P value
CTX	-0.937	0.001*				
BMD	0.937	0.001*	-0.999	0.001*		
BMD-Z score	0.932	0.001*	-0.984	0.001*	0.989	0.001*

the blood. Obesity has also been shown to reduce 25(OH)vitamin D production by upregulating the expression of enzymes that negatively influence vitamin D metabolism. Parathyroid hormone levels rise when 25(OH) vitamin D levels drop below 30 ng/mL.[27] Nevertheless, other studies showed that leptin is the cause of hyperparathyroidism since it affects PTH either via endocrine or paracrine means.[22,28]

Bone is a dynamic organ that is always changing and reshaping. Osteoblasts generate new bone, while osteoclasts break down existing bone.[26] Therefore, bone health is impacted by a disparity between bone creation and resorption at any time.[29]

Obesity causes bone marrow stem cells to differentiate into adipocytes, increasing bone marrow cavities. As a result, bone fragility increases, and bone microcirculation decreases. Second, visceral fat is assumed to be an endocrine organ that secretes adipokines and cytokines such as interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α). There has been widespread agreement that IL-6 and TNF-α increase bone loss by enhancing osteoclast generation.[5] Finally, physical activity is a fundamental physical trigger for bone formation, but obese children tend to lead less active lifestyles.[30-33]

Many studies, including ours, found that CTX levels were higher in obese children. PINP, on the other hand, was not significantly different between groups. The authors hypothesized that leptin would speed up bone degradation in obese subjects, resulting in a failure to gain bone mass efficiently.[34,35]

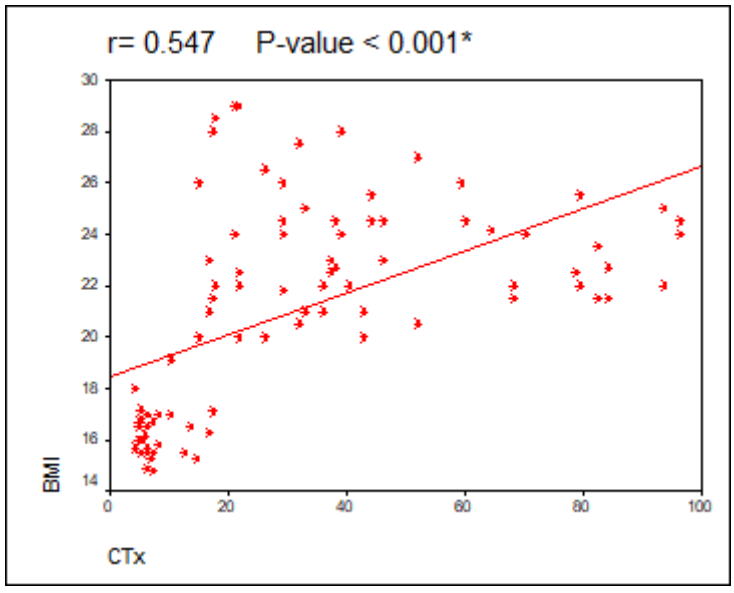

Figure 1. Positive correlation between BMI and CTX in the case group

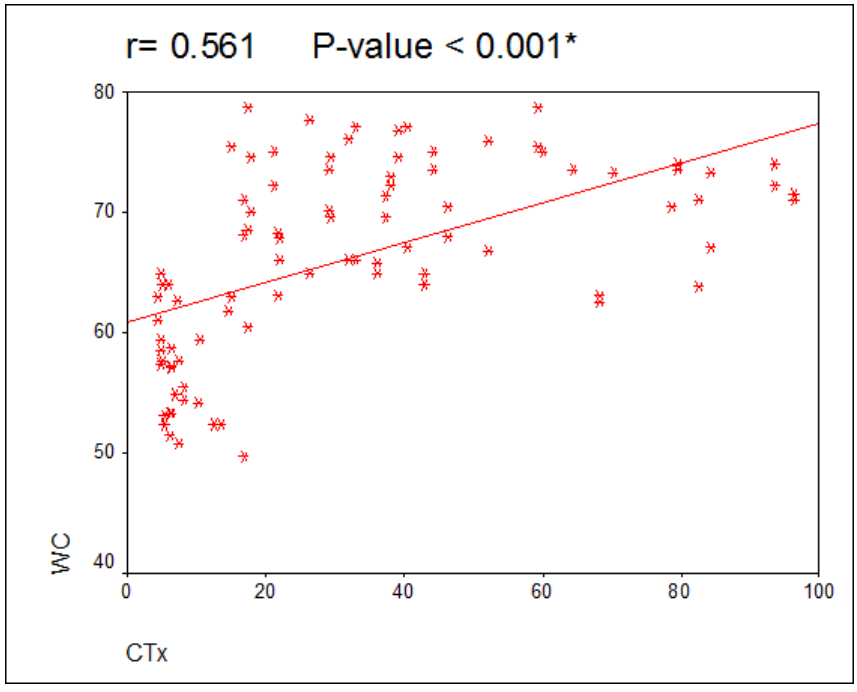

Figure 2. Positive correlation between WC and CTX in the case group

We found that our case group had significantly lower BMD and BMD Z-scores than the normoweight control group. Similarly, a previous study discovered that children had lower whole-body and lumbar spine BMD with increasing percent body fat and total fat mass, which was consistent with our findings.[36] Another study discovered an important link between normoweight and higher bone density Z-scores compared to overweight and underweight subjects. The overweight and underweight ones

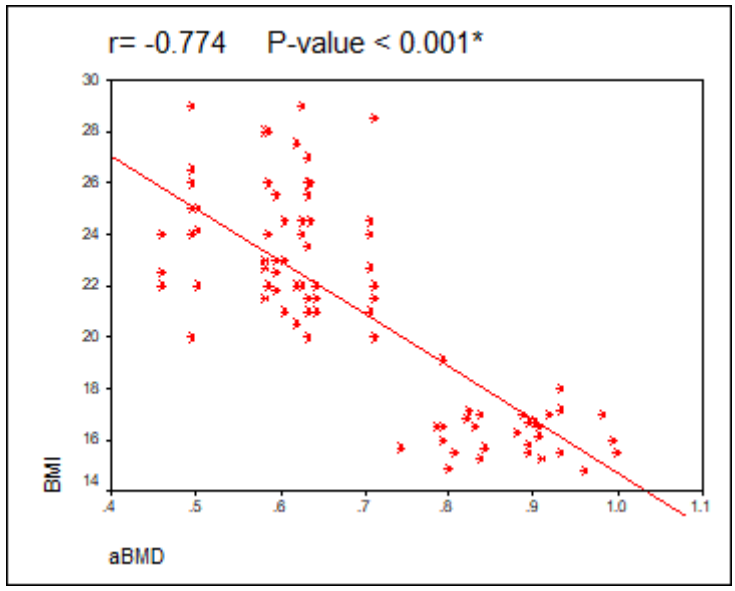

Figure 3. Negative correlation between BMI and BMD in the case group

had considerably lower BMD Z-scores than the normoweight group. This was explained by both extremes of weight are considered a significant risk factor for the development of low BMD in children.[37]

Overall, our findings imply that obesity during skeletal growth periods (particularly bone accretion) may disrupt skeletal adaptation to mechanical stresses. Fat, which alters hormonal variables and cytokines throughout development, may have a critical role in disrupting bone accretion.[38-41] This could also be linked to a lack of physical activity and nutritional imbalance.[42,43]

Many studies, on the other hand, determined that prepubertal children with obesity had considerably greater BMD than normoweight children, as measured by total body less head bone mass density (TBLH-BMD), TBLH-BMD Z-score, and BMD Z-score at L2-L4.[44-47] A study discovered that obese patients had higher BMD and BMD Zscore because of enhanced bone production with decreased bone loss.[29] The higher BMD findings in obese children in these studies may be related to inclusion criteria, study design, bone measurement procedures, and data analysis differences. It is also challenging to locate appropriate controls since obese children are often taller than normoweight subjects. Pubertal spikes and growth rates have been reported to be important factors in other study.[48] We cannot provide clear conclusions because there is no gold standard methodology for estimating BMD in these patients with increasing body size. Interactions between race and gender may also explain some of the study's conflicting results. Finally, all studies were limited by small sample sizes.

The DEXA scan is widely utilized in pediatric applications as suggested by the International Society for Clinical Densitometry, because it analyzes extensive areas of concern quickly at relatively low radiation doses and is resistant to mobility and posture alteration.[49,50]

In our obese prepubertal case group, there were no significant differences in PINP, CTX, BMD, or BMD Z-score between males and females in the case group. In contrast, Sabre et al. reported that CTx was significant lower in obese females compared to obese boys (P=0.018).[22] These gender disparities can be complex, as differences may present in pubertal state, body composition, hormonal environment, and adipose tissue distribution.[36]

According to our study, WC, as a measure of visceral and central obesity, as well as BMI and BMI Z-score, had significant positive associations with CTX, and significant inverse associations with PINP, BMD, and BMD Z-score. A few studies have found that fat mass, BMI, and weight have inverse relationships with lumbar BMD in obese adults and children. Furthermore, bone health responds more to muscle contractions than to sedentary loads, which

are reflected by body weight and fat mass.[51]

Bone marrow density estimates were greater in normoweight and overweight groups than in obese and very obese groups. Logistic analysis revealed that the relationship between BMD and WC was inverse. They hypothesized that this rise in BMD in overweight children was due to the body's compensation mechanism in response to weight gain, but in obese children, this mechanism failed, resulting in a large decline in BMD. So, it is worth noting that WC as an indicator of trunk fat is the most suitable and straightforward marker for clinical practice.[52] A study reported that weight, lean mass, fat mass, and BMI were all significantly associated with whole-body bone mineral content, whole-body BMD, and lumbar spine BMD.[53] The discrepancies in various studies may be attributed to differences in ethnic groups, age, weight, or height, physical activity, smoking habits, duration of sun exposure, and other factors that may affect BMD.[54]

In conclusion, BMD is decreased in prepubertal obese children, so obesity may have a negative impact on bone health. Furthermore, low BMD was associated with high CTX in prepubertal obese children, especially those with high visceral obesity. Thus, determining a line of treatment to decrease bone resorption may be prudent for obese prepubertal children.

Conflict of interest

None declared.

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