# Relationship Between Bone Mineral Density and Related Factors in Puberty: a 4-year Longitudinal Survey

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## Abstract

Bone fragility is a major factor in frail locomotive syndrome. For this fundamental measure, it is important to understand the changes in puberty, where bone metabolism is most active.The subjects were 277 boys and girls who participated in the Iwaki Health Promotion Project from 2011 to 2015 in both the fifth grade (10th/11th y.o) and the third grade of junior high school (14th/15th y.o). Bone mineral density and bone metabolism-related factors (physique and bone metabolism marker) during 4 years of puberty were examined. As for the physique at $10^{th}/11^{th}$ y.o, all factors were correlated with the bone density at $14^{th}/15^{th}$ y.o in boys and in girls, height, body muscle ratio, and grip strength value were correlated. In the 4-year rate of change, there was a positive correlation between height, body muscle rate, and bone mineral density in boys, but not in girls. For both boys and girls, a positive correlation was found between the physique (height, body muscle ratio, grip strength) in $10^{th}/11^{th}$ y.o, bone density at $14^{th}/15^{th}$ y.o, and bone metabolism markers.It is important to increase the peak bone mass: and prevent bone fragility by building a sufficient physique for boys ($14^{th}/15^{th}$ y.o) and girls ($10^{th}/11^{th}$ y.o).

## Introduction

In recent years, the prevention and treatment of frailty syndrome and locomotive syndrome have attracted attention due to changes in lifestyle and the advent of an aging society.

Bone fragility is a major factor in frailty syndrome or locomotive syndrome.

In general, bone resorption and formation are in balance after the end of skeletal growth. However, it loses its balance after the menopause (females) or in their 60s or 70s (in males) due to the abnormalities in metabolic regulators (aging, sex hormones, dyslipidemia, oxidation, glycation etc.)

In addition to the conventional bone mineral density (BMD) measurement, bone metabolism markers are used for assessing bone fragility. BMD measurement and bone metabolism marker measurement are means of observing two different aspects of bone strength. According to the statement at the NIH consensus conference 1), in terms of osteoporosis, these two factors are independent bone strength-related indicators, and are not related to each other. In addition, the FIT subanalysis showed that the markers that better reflect fracture risk have been found to be the bone formation markers rather than the bone resorption markers.

Observing the changes from childhood to adulthood, it has been determined that bone density (bone mass) reaches its maximum value (peak bone mass: PBM) between puberty and approximately 20 years of age. Since puberty is the age where the secondary sexual characteristics develop, changes in bone metabolism and physique are prominent due to increased sex hormones as the bone metabolism regulators. Therefore, there can be no fundamental countermeasure for the frail locomotive syndrome without understanding their relationships. Also, there have been only a few researches in the past, and none of them have considered the bone metabolism markers in their studies. This was due to the difficulty in targeting a large number of subjects in that particular age range, as well as a limited availability of efficient technique to measure bone quality, density, metabolism status and body composition.

In recent years, the measurement of body composition has become more efficient by the use of impedance method, which allows measurements of body muscle ratio and fat mass for each body part. In addition, an ultrasonic method has also been developed for the measurement of bone density, which is widely used nowadays. Both methods are simple to operate, have little invasiveness and few side effects, and can measure a large number of subjects.

In this study, bone mineral density, physique, and bone metabolism markers were measured over the period of 4 years in puberty in Aomori Prefecture. In addition to changes in bone mineral density and bone metabolism during puberty, their effects on the body were also measured, and factors that have influences on diseases were investigated.

The features of this study are as follows.

1. This is a 4-year cohort study that was carried out on puberty from the fifth grade of elementary school (10th /11th y. o.) to the third grade of junior high school (14th /15th y. o.).
2. Bone metabolism markers were measured in the third grade of 10th /11th y. o.
3. Body composition, body muscle ratio, and fat mass were examined separately.

## Subjects And Methods

### 1. Subjects

The subjects were 323 boys and girls who participated in the Elementary and Junior High School Health Survey of the Iwaki Health Promotion Project from 2011 to 2015 in both the fifth grade (10th /11th y. o) and the third grade of junior high school (14th /15th y. o.). After excluding those with a history of cardiovascular disease, diabetes mellitus, dyslipidemia, and hypertension, those with fractures, and those with deficient values, data from total of 277 puberty (125 boys and 152 girls) were analyzed for the study. In addition, 1,050 adults (438 males and 612 females) aged 20–89 years who participated in the Iwaki Health Promotion Project in 2018 were also included to make a comparison of the bone metabolism markers.

## 2. Methods

(1) Questionnaire survey

A self-administered questionnaire was distributed to the subjects prior to the investigation day. The answers to the questionnaire were confirmed by interviews and were collected on the day of measurement. Question items included participants' age, gender, past medical history, current medical history, and medication status.

(2) Blood pressure, body physique and body composition

The height, body weight, blood pressure and body composition were measured on the day of the survey.

The body weight, body fat percentage, and body muscle percentage were used to derive the body composition. A body composition analyzer (TBF-110, Tanita Corporation, Tokyo) that followed the bioelectrical impedance method was used for the measurement. This device uses multiple frequencies, such as 5 kHz, 50 kHz, 250 kHz, and 500 kHz, and is widely used in research targeting adults. In addition, the measuring instrument is intended for the analysis of the body composition of subjects with a height of 110 cm and an age of 6 years or older. It has also been reported that this measurement method is highly correlated with the DXA method.

(3) Bone evaluation

The bone evaluation was performed with an ultrasonic bone evaluation device (AOS-100NW, Aroca Co., Ltd., Tokyo) using a quantitative ultrasonic method. The measurement site was the calcaneus, and the evaluation was based on the acoustic bone evaluation value (Osteo Sono-assessment Index; OSI). With this device, the ultrasonic propagation speed (Speed of Sound: SOS) and the ultrasonic transmission index (TI) were measured, and the OSI was calculated.

SOS corresponds to calcification and bone density, and TI corresponds to broadband ultrasound attenuation (BUA), which is a bone quality evaluation index, and reflects trabecular bone and bone density. OSI is calculated by the arithmetic expression: $OSI = TI \times SOS^2$, which reflects the characteristics of both SOS and TI. Therefore, OSI is regarded as one of the comprehensive index values in acoustic bone evaluation.

(4) Physical fitness

Regarding the physical strength, we measured the grip strength that is known to reflect the muscular strength of the whole body. It was conducted according to the method of the “New Physical Strength Test” established by the Japan Sports Agency of the Ministry of Education, Culture, Sports, Science and Technology.

The Smedley type grip strength meter was used to measure the grip strength, and the grip width was adjusted so that the proximal interphalangeal joint of the second finger was flexed at 90°. For measurement, the limb position was a standing position with both legs open, the pointer of the grip strength meter was held so that it faced outside, and the shoulders were slightly laterally displaced so as not to touch the body, and then the subject was asked to grasp the grip strength meter with full force while exhaling. During the measurement, subjects were made sure that the grip strength meter was held still while they maintained the basic posture. The measurement was performed twice on each leg, and measurements were taken alternately on the left and right legs, and the best value was adopted. The measurements were taken twice alternately on each leg, and the highest value was recorded as the result.

(5) Blood biochemical parameter

The blood samples were taken from children in the third grade of junior high school (14th /15th y. o) and adults in the early morning on an empty stomach. The blood analysis was outsourced to LSI Medience Co., Ltd. The company also conducted the measurements of bone formation markers (bone-type alkaline phosphatase, BAP, CLEIA method) and bone resorption markers (type I collagen cross-linking NNTX, CLEIA method).

## 3. Statistical analysis method

All analyses were performed by gender.

The items obtained from the fifth grade of elementary school (10th /11th y.o.) and those from the third grade of junior high school (14th /15th y. o.) were compared using the paired t-test.

In order to examine the potential confounding factors, the correlations between OSI and height, body fat percentage, body muscle percentage, and grip strength was confirmed for each grade using a partial correlation coefficient. At 14th /15th y.o., the correlation between OSI, BAP, and NTX was confirmed using the partial correlation coefficient for 14th /15th y. o.

For investigating the effect of changes in physique and physical strength on OSI from the fifth grade of elementary school (10th /11th y. o.)to the third grade of junior high school (14th /15th y. o.), the changes in body fat percentage (%fat), body muscle percentage (% muscle), and grip strength with respect to the change in OSI were examined using the multiple regression analysis. For the multiple regression analysis, the amount of change in OSI was used as the objective variable, and the amount of change in each item was used as the explanatory variable.

The odds ratio and its 95% confidence interval were calculated using the logistic regression analysis to assess the relationship between physique and physical parameters in 10th /11th y. o and bone metabolism (OSI, BAP, and NTX) in 14th /15th y. o.

Height, % fat, % muscle, and grip strength were used as physique / physical fitness indicators for 10th /11th y. o., and each was divided into tertile. (low value group, medium value group and high value group).

The SPSS 16.0J software (IBM, SPSS Inc., Chicago, IL, USA) was used for statistical analysis, and the significance level was set to be $P < 0.05$.

**4. Ethical considerations**

The purpose of the study, the right to withdraw from the study at any point, the data management method including privacy data protection and data anonymity were thoroughly explained to all subjects, and written consents were obtained from either themselves or from their parents/carers.

The Iwaki Health Promotion Project Elementary and Junior High School Health Survey was approved by the Institutional Review Board of the Graduate School of Medicine, Hirosaki University (ethics numbers: 2009-048, 2010-084, 2011 – 111, 2012 – 163, 2013 – 339, 2014-060, and 2015-075) and conducted in accordance with the principles of the Declaration of Helsinki.

This study was registered with UMIN (examination name: Iwaki Health Promotion Project Medical Examination; UMIN Exam ID: UMIN000040459).

# Results

1. Differences between 10th /11th y.o. and 10th /11th y.o. (Tables 1 and 2)

In boys, all items except body fat percentage was significantly increased (all $P = 0.000$), whereas in girls, all items were increased significantly ($P = 0.000$).

Table 1
Characteristics of subject (boys, N = 125)

| | **10th /11th y. o.** | **14th /15th y. o.** | ***P-value*** |
|---|---|---|---|
| Height (cm) | 142.5 ± 6.7 | 167.8 ± 6.8 | *0.000* |
| Body weight (kg) | 37.0 ± 9.4 | 57.7 ± 10.9 | *0.000* |
| % fat (%) | 17.2 ± 10.8 | 17.9 ± 7.7 | *0.160* |
| % muscle (%) | 28.3 ± 3.6 | 44.2 ± 4.8 | *0.000* |
| Hand grip (kg) | 17.8 ± 3.4 | 32.1 ± 5.4 | *0.000* |
| OSI ($\times 10^5$) | 2.6 ± 0.4 | 2.9 ± 0.4 | *0.000* |
| BAP (μg/L) | - | 57.6 ± 38.7 | - |
| NTX (nmol BCE/mmol) | - | 77.7 ± 31.5 | - |
| Mean ± Standard deviation, Pared t test | | | |

Table 2
Characteristics of subject (girls, N = 152)

| | **10th /11th y. o.** | **14th /15th y. o.** | ***P-value*** |
|---|---|---|---|
| Height (cm) | 144.5 ± 6.7 | 157.2 ± 5.7 | *0.000* |
| Body weight (kg) | 37.4 ± 7.4 | 51.7 ± 8.3 | *0.000* |
| % fat (%) | 20.0 ± 7.1 | 30.2 ± 6.7 | *0.000* |
| % muscle (%) | 27.9 ± 3.5 | 33.6 ± 3.2 | *0.000* |
| Hand grip (kg) | 17.6 ± 3.3 | 22.6 ± 3.9 | *0.000* |
| OSI ($\times 10^5$) | 2.5 ± 0.2 | 3.0 ± 0.4 | *0.000* |
| BAP (μg/L) | - | 23.0 ± 15.5 | - |
| NTX (nmol BCE/mmol) | - | 35.4 ± 13.8 | - |
| Mean ± Standard deviation, Pared t test | | | |

2. Correlation in each grade (Tables 3 and 4)

No significant correlation was found between each related item and OSI in 10th /11th y. o..

Table 3
Correlation coefficients between bone density and other parameters (boys, N = 125)

| | | **Height** | | **%fat** | | **%muscle** | | **Hand grip** | | **OSI** | | **BAP** | | **NTX** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | R | *P-value* | R | *P-value* | r | *P-value* | R | *P-value* | R | *P-value* | R | *P-value* | R | *P-value* |
| 10th /11th y. o. | Height | | | 0.287 | *0.001* | 0.886 | *0.000* | 0.451 | *0.000* | 0.075 | *0.204* | | | | |
| | % fat | 0.287 | *0.001* | | | 0.485 | *0.000* | 0.348 | *0.000* | 0.058 | *0.259* | | | | |
| | % muscle | 0.886 | *0.000* | 0.485 | *0.000* | | | 0.594 | *0.000* | 0.080 | *0.186* | | | | |
| | Hand grip | 0.451 | *0.000* | 0.348 | *0.000* | 0.594 | *0.000* | | | 0.060 | *0.254* | | | | |
| | OSI | 0.075 | *0.204* | 0.058 | *0.259* | 0.080 | *0.186* | 0.060 | *0.254* | | | | | | |
| 14th /15th y. o. | Height | | | 0.060 | *0.252* | 0.732 | *0.000* | 0.363 | *0.000* | 0.148 | *0.049* | -0.445 | *0.000* | -0.406 | *0.000* |
| | % fat | 0.060 | *0.252* | | | 0.460 | *0.000* | 0.256 | *0.002* | 0.274 | *0.001* | -0.025 | *0.390* | -0.209 | *0.010* |
| | % muscle | 0.732 | *0.000* | 0.460 | *0.000* | | | 0.651 | *0.000* | 0.429 | *0.000* | -0.122 | *0.088* | -0.339 | *0.000* |
| | Hand grip | 0.363 | *0.000* | 0.256 | *0.002* | 0.651 | *0.000* | | | 0.390 | *0.000* | -0.142 | *0.057* | -0.454 | *0.000* |
| | OSI | 0.148 | *0.049* | 0.274 | *0.001* | 0.429 | *0.000* | 0.390 | *0.000* | | | -0.328 | *0.000* | -0.331 | *0.000* |
| | BAP | -0.025 | *0.390* | -0.122 | *0.088* | -0.142 | *0.057* | -0.328 | *0.000* | -0.445 | *0.000* | | | 0.366 | *0.000* |
| | NTX | -0.209 | *0.010* | -0.339 | *0.000* | -0.454 | *0.000* | -0.331 | *0.000* | -0.406 | *0.000* | 0.366 | *0.000* | | |
| Partial correlation coefficient. | | | | | | | | | | | | | | | |

Table 4
Correlation coefficients between bone density and other parameters (girls, N = 152)

| | | Height | | %fat | | %muscle | | Hand grip | | OSI | | BAP | | NTX | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | R | *P-value* | R | *P-value* | R | *P-value* | R | *P-value* | R | *P-value* | R | *P-value* | R | *P-value* |
| 10th /11th y. o. | Height | | | 0.207 | *0.005* | 0.885 | *0.000* | 0.659 | *0.000* | 0.405 | *0.000* | | | | |
| | % fat | 0.207 | *0.005* | | | 0.463 | *0.000* | 0.130 | *0.055* | 0.188 | *0.010* | | | | |
| | % muscle | 0.885 | *0.000* | 0.463 | *0.000* | | | 0.672 | *0.000* | 0.525 | *0.000* | | | | |
| | Hand grip | 0.659 | *0.000* | 0.130 | *0.055* | 0.672 | *0.000* | | | 0.356 | *0.000* | | | | |
| | OSI | 0.405 | *0.000* | 0.188 | *0.010* | 0.525 | *0.000* | 0.356 | *0.000* | | | | | | |
| 14th /15th y. o. | Height | | | -0.095 | *0.242* | 0.781 | *0.000* | 0.532 | *0.000* | 0.154 | *0.058* | -0.075 | *0.359* | 0.093 | *0.255* |
| | % fat | -0.095 | *0.242* | | | 0.210 | *0.009* | 0.071 | *0.385* | 0.157 | *0.054* | -0.178 | *0.028* | -0.202 | *0.013* |
| | % muscle | 0.781 | *0.000* | 0.210 | *0.009* | | | 0.619 | *0.000* | 0.374 | *0.000* | -0.097 | *0.236* | -0.094 | *0.250* |
| | Hand grip | 0.532 | *0.000* | 0.071 | *0.385* | 0.619 | *0.000* | | | 0.242 | *0.003* | -0.137 | *0.093* | -0.061 | *0.455* |
| | OSI | 0.154 | *0.058* | 0.157 | *0.054* | 0.374 | *0.000* | 0.242 | *0.003* | | | -0.067 | *0.411* | -0.189 | *0.020* |
| | BAP | -0.075 | *0.359* | -0.178 | *0.028* | -0.097 | *0.236* | -0.137 | *0.093* | -0.067 | *0.411* | | | 0.376 | *0.000* |
| | NTX | 0.093 | *0.255* | -0.202 | *0.013* | -0.094 | *0.250* | -0.061 | *0.455* | -0.189 | *0.020* | 0.376 | *0.000* | | |

Partial correlation coefficient.

On the other hand, there was a significant positive correlation between all related items and OSI in girls (height: *P* = 0.000, body fat percentage: *P* = 0.010, body muscle percentage: *P* = 0.000, and grip strength: *P* = 0.000).

OSI was positively correlated with body fat percentage, height, body muscle percentage and grip strength in the boys at 14th /15th y. o. (*P* = 0.049, *P* = 0.001, *P* = 0.000, *P* = 0.000, and *P* = 0.000, respectively), whereas it was negatively correlated with BAP and NTX (*P* = 0.000 for both). Height was positively correlated with body muscle ratio, grip strength, and OSI (*P* = 0.000, *P* = 0.000, and *P* = 0.049, respectively) and negatively correlated with NTX values (*P* = 0.010). Body fat percentage was positively correlated with body muscle percentage, grip strength, and OSI (*P* = 0.000, *P* = 0.000, and *P* = 0.049, respectively) and negatively correlated with NTX (*P* = 0.010). Body muscle percentage was positively correlated with height, body fat percentage, grip strength, and OSI (all *P* = 0.000) and negatively correlated with NTX (*P* = 0.010). Grip strength was positively correlated with height, body fat percentage, body muscle percentage, and OSI (*P* = 0.000, *P* = 0.002, *P* = 0.000, and *P* = 0.000, respectively) and negatively correlated between BAP and NTX (all *P* = 0.000). BAP values were negatively correlated with height and OSI (all *P* = 0.000) and positively correlated with NTX (*P* = 0.000). NTX had a negative correlation with height, body fat percentage, body muscle percentage, grip strength, and OSI (*P* = 0.000, *P* = 0.010, *P* = 0.000, *P* = 0.000, and *P* = 0.000, respectively) and had a positive correlation with BAP (*P* = 0.000).

For the girls in 14th /15th y. o. a positive correlation was found between OSI, body muscle ratio, and grip strength (*P* = 0.000, *P* = 0.003, respectively). Height was positively correlated with and body muscle percentage and grip strength (*P* = 0.000 and *P* = 0.000, respectively). Body fat percentage was positively correlated with body muscle percentage (*P* = 0.009) and negatively correlated with BAP and NTX (*P* = 0.028 and *P* = 0.013, respectively). Body muscle percentage was positively correlated with height, body fat percentage, grip strength, and OSI (*P* = 0.000, *P* = 0.009, *P* = 0.000, and *P* = 0.000, respectively). Grip strength was positively correlated with height, body muscle ratio, and OSI (*P* = 0.000, *P* = 0.000, and *P* = 0.003, respectively). BAP was negatively correlated with body fat percentage (*P* = 0.028) and positively correlated with NTX (*P* = 0.000). NTX showed a negative correlation with body fat percentage and OSI (*P* = 0.013 and P = 0.020, respectively) and a positive correlation with BAP (*P* = 0.000).

3. Relationship between changes of related items and bone density over the 4 years (Tables 5 and 6)

For boys, the amount of change in height and the amount of change in body muscle rate affected the amount of change in OSI (*P* = 0.000 and *P* = 0.001 respectively). For girls, there were no such factors influencing the amount of change in OSI.

Table 5
Results of multiple regression analysis (boys, N = 125)

| Objective valuable | Explanatory variable | Partial regression coefficient (B) | Standard error | standardized partial regression coefficient(β) | *P-value* | Adjusted $R^2$ |
|---|---|---|---|---|---|---|
| OSI | Changing value of height | -0.061 | 0.013 | -0.490 | *0.000* | 0.567 |
| | Changing value of % fat | 0.001 | 0.008 | 0.014 | *0.879* | |
| | Changing value of % muscle | 0.058 | 0.017 | 0.344 | *0.001* | |
| | Changing value offhand grip | 0.009 | 0.009 | 0.072 | *0.310* | |
| Multiple regression analysis. Adjusted by values at the 5th grade of primary school. | | | | | | |

Table 6
Results of multiple regression analysis (girls, N = 152)

| Objective valuable | Explanatory variable | Partial regression coefficient (B) | Standard error | standardized partial regression coefficient(β) | *P-value* | Adjusted $R^2$ |
|---|---|---|---|---|---|---|
| OSI | Changing value of height | -0.010 | 0.012 | -0.173 | *0.411* | -0.010 |
| | Changing value of % fat | 0.004 | 0.006 | 0.069 | *0.480* | |
| | Changing value of % muscle | 0.023 | 0.021 | 0.210 | *0.288* | |
| | Changing value offhand grip | 0.013 | 0.010 | 0.140 | *0.187* | |
| Multiple regression analysis. Adjusted by values at the 5th grade of primary school. | | | | | | |

4. The influence which health state at 10th /11th y. o. have in 4 years when they are at10th /11th y. o. (Tables 7 and 8)

(1) Influence on OSI: Boys showed high odds ratios in the group with high height, body fat percentage, and grip strength ($P$ = 0.003, = 0.004, and = 0.001, respectively). In terms of body muscle ratio, the medium-value group and the high-value group showed significantly higher odds ratios ($P$ = 0.015 and $P$ = 0.000, respectively). For girls, the odds ratios for height, body muscle percentage, and grip strength were significantly higher in the medium- and high-value groups (height: $P$ = 0.001 and $P$ = 0.001, body muscle percentage: $P$ = 0.004 and $P$ = 0.000, and grip strength: $P$ = 0.037 and $P$ = 0.006, respectively), but there was no significant difference in body fat percentage.

(2) Influence on BAP: In boys, the odds ratio was significantly higher in the high body fat percentage and body muscle percentage groups ($P$ = 0.008 and $P$ = 0.002, respectively). In the case of grip strength, the odds ratios were significantly higher in the medium- and high-value groups ($P$ = 0.008 and $P$ = 0.000, respectively). However, there was no significant difference in height. Girls showed significantly higher odds ratios in the high body fat percentage and grip strength groups ($P$ = 0.002 and $P$ = 0.003, respectively). In terms of height and body muscle ratio, significantly higher odds ratios were shown in the medium- and high-value groups (height: $P$ = 0.047 and $P$ = 0.001 and body muscle ratio: $P$ = 0.004 and $P$ = 0.001, respectively).

(3) Influence on NTX: In boys, the odds ratio was significantly higher in the group with high height, body fat percentage, and grip strength ($P$ = 0.000, $P$ = 0.000, and $P$ = 0.004, respectively). In terms of body muscle ratio, the odds ratios were significantly higher in the medium and high-value groups ($P$ = 0.033 and $P$ = 0.000, respectively). Girls showed significantly higher odds ratios in all the medium- and high-value groups (height: $P$ = 0.014 and $P$ = 0.002, body fat percentage: $P$ = 0.021 and $P$ = 0.013, body muscle percentage: $P$ = 0.001 and $P$ = 0.000, and grip strength: $P$ = 0.040 and $P$ = 0.000, respectively).

Table 7

Odd ratios of body physic / physical fitness at 10th /11th y. o. for bone metabolic markers at 14th /15th y. o. (boys, N = 125)

| | | Trixie(N) | OSI | | | BAP | | | NTX | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | β | 95%CI | *P-value* | β | 95%CI | *P-value* | β | 95%CI | *P-value* |
| 5th grade at primary school | Height(cm) | 126.7-139.5(41) | - | - | - | - | - | - | - | - | - |
| | | 139.6–145.0(42) | 1.841 | [0.500-6.771] | *0.340* | 1.004 | [0.993–1.015] | *0.520* | 0.993 | [0.979–1.007] | *0.300* |
| | | 145.1-158.5(42) | 7.057 | [1.952–25.506] | *0.000* | 0.989 | [0.978–1.001] | *0.080* | 0.963 | [0.945–0.981] | *0.000* |
| | % fat(%) | 3.0-10.3(41) | - | - | - | - | - | - | - | - | - |
| | | 10.5–18.8(41) | 1.612 | [0.485–5.356] | *0.170* | 0.993 | [0.982–1.004] | *0.230* | 1.000 | [0.987–1.014] | *0.990* |
| | | 19.0–57.0(41) | 3.387 | [3.387–10.872] | *0.040* | 0.984 | [0.972–0.996] | *0.010* | 0.965 | [0.947–0.984] | *0.000* |
| | % muscle(%) | 20.85–26.90(32) | - | - | - | - | - | - | - | - | - |
| | | 26.95–29.45(44) | 6.095 | [1.412–26.310] | *0.020* | 0.997 | [0.986–1.008] | *0.590* | 0.984 | [0.969–0.999] | *0.030* |
| | | 29.50–40.90(49) | 24.581 | [5.350-112.929] | *0.000* | 0.980 | [0.967–0.992] | *0.000* | 0.945 | [0.924–0.967] | *0.000* |
| | Hand grip(kg) | 9.4–15.9(32) | - | - | - | - | - | - | - | - | - |
| | | 16.0–18.0(44) | 1.570 | [0.356–6.923] | *0.550* | 0.981 | [0.967–0.995] | *0.010* | 0.993 | [0.979–1.007] | *0.310* |
| | | 19.0–28.0(49) | 10.764 | [2.505–46.260] | *0.000* | 0.969 | [0.955–0.984] | *0.000* | 0.977 | [0.962–0.993] | *0.000* |

Logistic regression analysis, standardized partial regression coefficient: β

Table 8
Odd ratios of body physic / physical fitness at 10th /11th y. o. for bone metabolic markers at 14th /15th y. o. (girls, N = 152)

| | | Trixie(N) | OSI | | | BAP | | | NTX | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | β | 95%CI | *P-value* | β | 95%CI | *P-value* | β | 95%CI | *P-value* |
| 5th grade at primary school | Height(cm) | 127.9-141.9(54) | - | - | - | - | - | - | - | - | - |
| | | 142.1-147.4(49) | 10.349 | [2.761–38.792] | *0.000* | 0.973 | [0.947-1.000] | *0.050* | 0.962 | [0.933–0.992] | *0.010* |
| | | 147.5-163.3(49) | 9.789 | [2.616–36.629] | *0.000* | 0.944 | [0.913–0.975] | *0.000* | 0.945 | [0.912–0.979] | *0.000* |
| | % fat(%) | 8.6–16.5(51) | - | - | - | - | - | - | - | - | - |
| | | 16.6–20.5(50) | 0.904 | [0.312–2.623] | *0.850* | 0.974 | [0.949–1.001] | *0.060* | 0.965 | [0.936–0.995] | *0.020* |
| | | 20.6–47.5(51) | 1.099 | [0.387–3.120] | *0.860* | 0.953 | [0.924–0.983] | *0.000* | 0.961 | [0.931–0.991] | *0.010* |
| | % muscle(%) | 18.55–26.20(50) | - | - | - | - | - | - | - | - | - |
| | | 26.30-29.35(48) | 8.010 | [1.980–32.410] | *0.000* | 0.956 | [0.926–0.986] | *0.000* | 0.944 | [0.912–0.978] | *0.000* |
| | | 29.50–39.10(54) | 19.066 | [4.666–77.911] | *0.000* | 0.948 | [0.918–0.979] | *0.000* | 0.934 | [0.901–0.969] | *0.000* |
| | Hand grip(kg) | 9.8–15.8(51) | - | - | - | - | - | - | - | - | - |
| | | 16.0–19.0(53) | 3.542 | [1.079–11.628] | *0.040* | 0.987 | [0.963–1.011] | *0.290* | 0.971 | [0.943–0.999] | *0.040* |
| | | 19.3–25.0(48) | 5.541 | [1.652–18.592] | *0.010* | 0.952 | [0.922–0.983] | *0.000* | 0.931 | [0.895–0.969] | *0.000* |

Logistic regression analysis, standardized partial regression coefficient: β

## Discussion

Bones repeats formation and resorption constantly throughout life. These bone metabolisms are regulated by various hormones and cytokines. Bone growth has two peaks: a primary growth phase, which occurs up to the age of 5 years, and a secondary growth phase in puberty. During the secondary growth period, bones finish growing in the longitudinal direction, the epiphyseal line is closed, the cancellous bone becomes denser, and the bone density increases. The age of the subjects in this study are of the ages required for the secondary sexual characteristics. Also, this is the age where bone metabolism regulators are closely associated with each other due to the increase of sex hormones. Rauchenzauner et al. reported that the age and gender variabilities of the bone metabolism in adolescent are very high and that both BAP and the bone resorption marker RACP-5b decrease after 15 y. o. However, there are very few reports on trends in bone metabolism markers and related factors in puberty.

The results obtained in this study suggest the followings;

1. For girls, the size of the physique at 10th /11th y. o. was found to have a positive correlation with the OSI at that time. However, this tendency was not seen in boys. This suggests that in girls, developing physique in early puberty is effective in acquiring OSI.
2. By examining the amount of change from 10th /11th y. o. to 14th /15th y. o., it was shown that the amount of change in height and body muscle ratio was positively correlated with the amount of change in OSI not only in boys, but also in girls. In addition, the amount of changes in all physique factors for the boys at 14th /15th y. o. was positively correlated with the amount of change in OSI. On the other hand, this tendency was low for the girls, and the amount of change in body muscle ratio and muscle strength were the only two items that were positively correlated with the amount of change in OSI. From the data obtained above, it was suggested that the development of physique from 10th /11th y. o. to 14th /15th y. o. is effective for acquiring OSI in boys.
3. For both boys and girls, a positive correlation was shown between body size (height, muscle ratio, and grip strength) at 10th /11th y. o and between OSI and bone metabolism at 14th /15th y. o. This correlation was significant in girls. This suggests that development of physique (height, body muscle ratio, and grip strength) in early puberty is effective in increasing OSI.

The peak height velocity age (PHVA) during the secondary growth period in Japanese is 13 years for boys and 11 years for girls, and this growth rate is higher for boys. At the age of 11 years, subjects are at 14th /15th y. o., whereas at the age of 13 years, subjects are at 12nd /13rd y. o. The subjects of this study, i.e., girls at 10th /11th y. o. are at PHVA, whereas boys 10th /11th y. o. is in the early stages of growth. On the other hand, in 14th /15th y. o., boys are at PHVA completion phase, whereas girls are in the period of finishing the secondary growth period. For boys and girls, the growth period ends at the age of 17 and 15 years, respectively, along with the closure of the epiphyseal line and an increase in bone density. Considering this background and the results of this study, it is important to note that the girls tend to reach PHVA approximately 2 years earlier than the boys, thus, their PBM needs to be increased when they are in 10th /11th y. o. For the boys, their physique needs to be developed by the same grade in order to increase PBM.

In the previous studies that included subjects from toddler to adolescent to examine the bone metabolism markers have reported an increase in both bone resorption markers and bone formation markers. This was suggested to be the result of active bone metabolism.

There have been many studies on the relationship between bone metabolism and bone density in adults. Although there are reports with slightly different tendency for men, the results of previous studies are generally summarized as increases in bone metabolism markers during the growth period, followed by a decrease in their 40s and remain unchanged in the elderly.

In the Iwaki Health Promotion Project, The results on adolescent Project and the trends of bone metabolism markers obtained in the Iwaki Health Promotion were similar to those of previous studies. Specifically, both markers were high at 14th /15th y. o. for both boys and girls (boys: BAP 57.6 ± 38.7 and NTX 77.7 ± 31.5; girls: BAP 23.0 ± 15.5 and NTX 35.4 ± 13.8), and then dropped sharply in adults (males in their early 20s: BAP 16.1 ± 3.7 and NTX 18.8 ± 4.4; females: BAP 12.2 ± 3.2 and NTX 14.9 ± 3.0), and remained stable thereafter. This indicated that bone metabolism is active in boys and girls at 14th /15th y. o. who have high values of both markers.

Both markers were higher in boys than in girls. Bone density increases after the epiphyseal line closes, leading to PBM. It was considered that there was a gender difference in boys at 14th /15th y. o. because they were still in the growth stage and the epiphyseal line was not closed, whereas in girls, it was the period when the epiphyseal line was closed. In other words, boys are in a period of height growth and bone growth is active, whereas girls are in a period of bone growth and bone maturation at 14th /15th y. o.

Based on the above data from the viewpoint of PHVA and for improving PBM, it is important to practice a lifestyle to increase height and muscle mass sufficiently by 14th /15th y. o. for boys and by 10th /11th y. o. for girls.

## Conclusions

The results were obtained by following children for 4 years from ages 10/11 to 14/15 living in the Iwaki district of Aomori prefecture.

The bone metabolism markers suggested that bone metabolism was prominently active at 14th /15th y. o., and this tendency was significantly remarkable in boys.

For girls, a significant relationship between physique and OSI was already observed in 10th /11th y. o., and it was considered important to develop physique at least by this time. On the other hand, for boys, the development of physique from 10th /11th y.o. to 14th /15th y. o. was considered to be important for increasing OSI. This difference is thought to be due to the fact that PHVA differs between men and women.

## Declarations

### Conflict of interest

There are no conflicts of interest (COI) to disclose regarding this study.

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### Authors' Contributions

A.A. designed the study, analyzed and interpreted the data. D.S., S.O., Y.K., H.S., S.T., K.M., and J.Y. contributed to data acquisition. S.N. contributed to the data interpretation. All authors reviewed and edited the manuscript and approved the final version.

### Data availability statement

The datasets used and/or analysed during the current study available from the corresponding author, S.N., upon reasonable request.

Correspondence and requests for materials should be addressed to S.N.

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## References

1. NIH Consensus Development Panel on Osteoporosis Prevention. Diagnosis and Therapy. JAMA2001; 285:785-95.
2. Bauer DC, Black DM, Garnero P, Hochberg M, Ott S,Orloff J, Thompson DE, Ewing SK, Delmas PD, Fracture Intervention Trial Study Group. Change in bone turnover and hip, non-spine and vertebral fracture in alendronate-treated women: the fracture intervention trial. J. BoneMiner Res. 2004; 19(8): 1250-1258.
3. WHO Scientific Group on the Prevention and Management of Osteoporosis (2000: Geneva, Switzerland) Prevention and management of osteoporosis. Report of a WHO scientific group. (WHO technical report series; 921)
4. Dimitri P, Wales JK, Bishop N. Fat and bone in children—differential effects of obesity on bone size and mass according to fracture history. J Bone Miner. Res. doi:10.1359/jbmr.090823.
5. A Pietrobelli, P Morini, N Battistini, G Chiumello, C Nuñez, SB Heymsfield. Appendicular skeletal muscle mass: prediction from multiple frequency segmental bioimpedance analysis. Eur j Clin Nutr.1998; 52:507-511.
6. Morita R, Yamamoto I, Yuu I, Hamanaka Y, Ohta T, Takada M, Matsushita R, Masuda K. Quantitative ultrasound for the assessment of bone status. Osteoporosis International.1997; 7:128-134.
7. Takase H, Sakane N, Morimoto S, Uchida T, Mori K, Katasima M, Katuragi N. Relationship between visceral fat accumulation and metabolic components by sex and age in workplace setting: A Japanese Study of Visceral Adiposity and Lifestyle Information-Utilization and Evaluation. Journal of Japan Society for the Study of Obesity.2020; 26(2):245-253.
8. Hans D, Njeh CF, Genant HK, Menuier PJ. Quantitative ultrasound in bone status assessment. Rev Rheumatol, 1998;65:489-498.
9. Terlizzi FD, Battista S, Cavani F, Cane V, Cadossi R. Influence of bone tissue density and elasticity on ultrasound propagation: an in vitro study. J Bone Miner Res, 2000; 15:2458-2466.
10. D Hans, C Wu, CF Njeh, S Zhao, P Augat, D Newitt, T Link, Y Lu, S Majumdar, HK Genant. Ultrasound velocity of trabecular cubes reflects mainly bone density and elasticity. Calcif Tissue Int, 1999; 64:18-23.
11. Glüer CC, Wu CY, Jergas M, Goldstein SA, Genant HK. Three quantitative ultrasound parameters reflect bone structure. Calcif Tissue Int, 1994; 55:46-52.
12. Langton CM, Palmer SB, Porter RW. The measurement of broadband ultrasound attenuation in cancellous bone. Eng Med, 1984; 13:89-91.
13. E Tsuda-Futami, D Hans, CF Njeh, T Fuerst, B Fan, J Li, YQ He, HK Genant. An evaluation of a new gel-coupled ultrasound device for the quantitative assessment of bone. The British journal of radiology 1999; 72:691-700.
14. Ikeda N, Murata S, Otao H, Murata J, Horie J, Mizota K. The Relationship between Grip Strength and Physical Function in Elderly Community-Dwelling Women [in Japanese]. Rigakuryoho Kagaku.2011; 26(2):255-258.
15. Anne E Wind, Tim Takken, Paul JM Helders, Raoul HH Engelbert. Is grip strength a predictor for total muscle strength in healthy children: puberty, and young adult? Eur J Pediatr, 2010; 169:281-287.
16. Japan Sports Agency. Physical fitness test. http://www.mext.go.jp/a_menu/sports/stamina/03040901.htm
17. Watanabe Y, Oshima H, Mizuno K, Sekiguchi C, Fukunaga M, Kohri K, Rittweger J, Felsenberg D, Matsumoto T, Nakamura T. Intravenous pamidronate prevents femoralbone loss and renal stone formation during 90day bed rest. J Bone Miner Res 2004; 19:1771-1778.
18. Fuchs RK, Bauer JJ, Snow CM. Jumping improves hip and lumbar spine bone mass in prepubescent children: a randomized controlled trial. J Bone Miner Res. 2001; 16(1):148-156.
19. Wang QJ, Suominen H, Nicholson PH, Zou LC, Alen M, Koistinen A, Cheng S. Influence of physical activity and maturation status on bone mass and geometry in early pubertal girls. Scand J Med Sci Sports.2005; 15(2):100-106.
20. Bilezikian JP, Morishima A, Bell J, Grumbach MM. Increased bone mass as a result of estrogen therapy in a man with aromatase deficiency. N Engl J Med. 1998; 339(9):599-603.
21. Kraemer WJ, Marchitelli L, Gordon SE, Harman E, Dziados JE, Mello R, Frykman P, McCurry D, Fleck SJ. Hormonal and growth factor responses to heavy resistance exercise protocols. J Appl Physiol.1990; 69(4):1442-1450.
22. Sutton JR, Coleman MJ, Casey J, Lazarus L. Androgen responses during physical exercise. Br Med J.1973; 1:520-522.
23. Chandani AK, Scariano JK, Glew RH, Clemens JD, Grarry PJ, Baumgatner RN. Bone mineral density and serum levels of amino-terminal propeptide and cross-linked N-telopeptides of type collagen in elderly men. Bone 2000; 26:513-518.
24. Markus R, Andrea S, Peter HE, Klaus K, Gerda F, Andrea G, Gerd F, Wolfgang H. Sex-and age-specific reference curves for serum markers of bone turnover in healthy children from 2 months to 18 years.J Clin Endocrinol Metab.2007; 92:442-449.
25. Scopacasa F, Wishart JM, Need AG, Horowitz M, Morris HA, Nordin BE. Bone density and bone-related biochemical variables in normal men:a longitudinal study. J Gerontol A Biol Sci Med 2002:385-391.
26. Ministry of Health, Labour and Welfare. Annual Report of School Health Statistics Research.https://www.e-stat.go.jp/stat-search/files?page=1&layout=datalist&toukei=00400002&tstat=000001011648&cycle=0&tclass1=000001098858&tclass2=000001098859&tclass3val=0
27. Fukunaga M. Bone Metabolism Markers.Tokyo: Medical Review; 2003:p114.
28. Garnero P, Sornay RE, Claustrat B, Delmas PD. Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: the OFELY study. J Bone Miner Res 2000; 15:1526-1536.

29. Miller PD, Hochberg MC, Wehren LE, Ross PD, Wasnich RD. How useful are measures of BMD and bone turnover? Curr Med Res Opin 2005; 21:545-554.
30. Szulc P, Garnero P, Munoz F, Marchand F, Delmas PD. Cross-sectional evaluation of bone metabolism in men. J Bone Miner Res 2001; 16:1642-1650.
31. Meier C, Nguyen TV, Center JR, Seibel MJ, Eisman JA. Bone resorption and osteoporotic fractures in elderly men: thedubbo osteoporosis epidemiology study. Bone Miner Res 2005; 20:579-587.
32. Szulc P, Delmas PD. Biochemical markers of bone turnover in men. Calcif Tissue Int 2001; 69:229-234.
33. Meier C, Liu PY, Handelsman DJ, Seibrl MJ. Endocrine regulation of bone turnover in men. Clin Endocrinol 2005; 63(6):603-616

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