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Association between bone turnover markers, leptin and nutritional status in girls with adolescent idiopathic scoliosis (AIS)

Edyta Matusik [1,*], Jacek Durmala [1], Magdalena Olszanecka-Glinianowicz [2], Jerzy Chudek [3], and Pawel Matusik [4,]

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[1] School of Health Sciences in Katowice, Department of Rehabilitation, Medical University of Silesia, Katowice, Poland; ematusik@wp.pl
[2] School of Medicine in Katowice, Health Promotion and Obesity Management Unit, Department of Pathophysiology, Medical University of Silesia, Katowice, Poland;
[3] School of Medicine in Katowice, Department of Internal Medicine and Oncological Chemotherapy, Medical University of Silesia, Katowice, Poland;
[4] School of Medicine in Katowice, Department of Pediatrics, and Pediatric Endocrinology, Medical University of Silesia, Katowice, Poland
* Correspondence: Dr. Edyta Matusik MD, PhD, Department of Rehabilitation, Medical University of Silesia, ul. Ziolowa 45/47, 40-065 Katowice, Poland ematusik@wp.pl ; Tel.: +48-32-3598241

Abstract: The link between scoliotic deformity and bone metabolism in adolescent idiopathic scoliosis (AIS) has not been well researched. Moreover, the data concerning the cross-talk between fat tissue content/hormonal activity and bone markers in this group of patients are lacking. The objective of this study was to correlate the extent of scoliotic-curve severity with the bone turnover vs. leptin level and nutritional status in girls with AIS. The study encompassed 77 AIS girls, aged 14.7 ± 2.17 years. Scoliotic curve severity assessed by Cobb's angle was categorized as mild (10-19º) moderate (20-39º) or severe (≥40º). Corrected height, weight, waist and hip circumferences were measured and body mass index (BMI), corrected height Z-score, BMI Z-score and waist/height ratio (WHtR) were calculated for the entire group. Body composition parameters: fat mass (FAT), fat-free mass (FFM) and predicted muscle mass (PMM) were determined using a bioelectrical impedance analyzer. Bone turnover markers (osteocalcin (OC) and amino terminal of collagen cross-links NTx) and leptin levels were assessed in serum. Multiple regression analysis showed that, OC, NTx (negatively with $p<0.05$) and leptin (positively with $p<0.01$) were significantly associated with curve severity in AIS girls. Moreover, Cobb's angle was positively correlated with W/HtR ($p<0.01$)and FAT ($p<0.05$). One-way analysis of variance (ANOVA) revealed significant differences in leptin ($p<0.05$ vs. mild only), OC ($p<0.05$ vs. mild and moderate)) and W/HtR ($p<0.01$ and $p<0.05$ vs. mild and moderate respectively) between the three AIS severity subgroups. OC was significantly lower in the severe AIS subgroup, while leptin and W/HtR were significantly higher. Significant correlations between leptin and anthropometrical parameters as BMI z-score and W/HtR were shown. Leptin level correlated also significantly with BMI z score ($p<0.001$), W/HtR ($p<0.0001$) and body composition parameters ($p<0.000001$). Moreover, there was a significant negative correlation between NTx and leptin level ($p<0.05$). Bone metabolism in AIS girls seems to be altered and significantly related to the scoliotic curve severity. Leptin may be a crucial link in the cross-talk between bone turnover and body composition in this group of patients. Further studies concerning this topic are needed.

Keywords: adolescent idiopathic scoliosis; bone turnover markers; leptin; body composition

1. Introduction

Adolescent idiopathic scoliosis (AIS) is the most common form of spinal deformity in the developmental period. The main clinical issue in this group of patients is the deformity progression. Pathogenesis of AIS probably has a multifactorial background [1-6] and is still under debate [7, 8]. Previous studies found that AIS is associated with low bone mineral density (BMD) and abnormal bone quality and strength [9-13], whilst the link between scoliotic deformity and bone metabolism in adolescent idiopathic scoliosis (AIS) has not been well researched and the exact mechanisms and causes of the bone loss in AIS are not identified yet. Recent study assessing bone mechanical properties in AIS patients showed that, osteopenia in this group of patients might be the result of abnormal regulation and modulation of bone metabolism and bone modeling/remodeling [13].

Leptin and its signaling pathway may be a candidate for the etiology of AIS. Leptin, together with the soluble leptin receptor (sOB-R), were shown to play an important role in the regulation of bone and energy metabolism in children. Leptin affects bone metabolism via central and peripheral ways. It modulates cortical bone formation by regulating the expression of several neuropeptides in hypothalamus and inducing sympathetic activation [14-17]. However, the data concerning the cross-talk between leptin level and bone markers in patients with AIS are lacking. Therefore, study showing the interrelationship between every scoliotic curve magnitude categories (mild, moderate, severe) in relation to the bone metabolism and leptin level is needed.

Accordingly, the aim of this study was to correlate the extent of scoliotic-curve severity with the bone turnover markers (osteocalcin (OC) and amino terminal of collagen cross-links (NTx)) vs leptin level and nutritional status in girls with AIS.

2. Materials and Methods

2.1 Studied population.

The study group comprised 77 newly-diagnosed AIS girls, aged 14.7 ± 2.17 years, recruited consecutively during their first visit to our Scoliosis Clinic at the Department of Rehabilitation. The diagnosis was confirmed by both, clinical assessment and standard standing postero-anterior X-ray film of the spine with Cobb's angle ≥10º. Subjects with a history of any forms of prior treatment for scoliosis, neuromuscular diseases, endocrine diseases, skeletal dysplasia, connective tissue abnormalities, glucocorticoid therapy, fractures, mental retardation or other congenital deformities were excluded from the study.

2.2 Scoliotic curve evaluation.

Scoliosis magnitude was evaluated by measuring Cobb's angle at the coronal plane of the whole spine on a standard X-ray film. In the case of double or triple scoliotic curves, the Cobb's angle of the major curve was selected. Curve severity was categorized as mild (Cobb's angle 10-19º) moderate (Cobb's angle 20-39º) or severe (Cobb's angle ≥40º) according to the conventional classification [18].

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2.3 Anthropometric measurements.

A set of anthropometric measurements was recorded at the first clinical visit. Standing height was measured by a wall-mounted Harpender Stadiometer to the nearest 0.1 cm. Weight (in underwear) was measured with an electronic scale with readings accurate to 0.1 kg. The corrected height was derived for every IS patient with Bjure's formula ($\log y = 0.011\ x\ -0.177$), where y is the loss of trunk height (cm) due to the deformed spine, and x is the greatest Cobb angle of the primary curve [19]. Body mass index (BMI) was then calculated, using the standard formula (kilograms per meter squared). Anthropometrical status was defined using BMI for age and sex, using the WHO percentile charts [20]. To adjust the anthropometrical status for age, BMI z -score, expressed as a number of standard deviations (SD) from the value of the 50th percentile, was calculated. BMI z -scores were derived using WHO AnthroPlus, version 1.0.4 (based on World Health Organization growth references) [20]. Waist circumferences were measured midway between the lower rib margin and the iliac crest in the standing position and waist to height ratio (W/HtR) was calculated.

2.4 Body composition analysis.

Body composition parameters: fat mass (FAT), fat-free mass (FFM), predicted muscle mass (PMM) and total body water (TBW) were assessed (in kilograms [kg] or as percentage of body weight [%]) based on bioelectrical impedance using a segmental body composition analyzer (BC-418MA Tanita Europe BV, Hoofddorp, The Netherlands).

2.5 Biochemical analysis.

Venous blood samples were drawn from antecubital vein in the morning in the supine position after the overnight fasting and collected in heparinized vacutainer tubes. After centrifugation at 1500 x g at 4^{o}C for 5 min, plasma was collected and transferred in Eppendorf™ tubes, then immediately frozen and stored at -80^{o}C until analysis. Competitive-inhibition enzyme-linked immunosorbent assay (ELISA) was used to evaluate amino terminal collagen cross-links (NTx) in serum (Osteomark NTx Serum). Quantitative sandwich enzyme immunoassay technique was used for the measurement of osteocalcin (OC) (MicroVue Osteocalcin EIA kit, Quidel, San Diego, USA) and leptin (TECOmedical AG, Swissach, Switzerland) . All samples were tested in duplicate.

2.6 Ethical considerations.

The study was approved by the Ethics Committee of the Medical University of Silesia. All participants and/or their caregivers gave informed consent. Patient rights were also approved according to the Helsinki Declaration.

2.7 Statistical analysis.

The following variables were not normally distributed (assessed by Kolmogorov-Smirnov test) and were log transformed to achieve near-normal distributions: leptin, osteocalcin, and NTx. Multivariate regression analysis adjusted to age and Tanner stage was performed to identify the variables that influence the curve severity expressed as Cobb's angle in the entire AIS population. Data are presented as the standardized regression coefficient (β) and adjusted r^2. One–way analysis of variance (ANOVA) was used to analyze any significant difference among the three curve magnitude subgroups, i.e. mild, moderate and severe. Correlations between continues parametrical (or log transformed) variables were based on linear Pearson's correlation coefficient. All statistical analysis was made with the Statistica™ 12 PL software and p value less than 0.05 was considered statistically significant.

3. Results

Baseline characteristics and anthropometric measurements of all studied girls are reported in table 1.

Multiple regression analysis using an age and Tanner stage adjustments was performed on the curve severity with respect to the bone markers, leptin and anthropometrical data. Bone turnover markers (OC and NTx) and leptin were found to be significantly and independently associated with curve severity in the studied AIS girls. Bone turnover seems to be negatively associated with the curve severity, while leptin level has a positive relation to the deformity magnitude. Moreover, Cobb's angle was positively correlated with W/HtR and FAT independently from age and Tanner stage. (Table 2).

For the next stage of the analysis, the study group was further divided according to the curve magnitude i.e. mild (10-19º) moderate (20-39º) or severe (≥40º). The mean Cobb's angles in the mild (n=36), moderate (n=30) and severe (n=11) groups were 19.96 ± 7.92º and 52.36 ± 12.54º, respectively.

One-way analysis of variance (ANOVA) revealed significant differences in leptin, osteocalcin and W/HtR between the three scoliotic severity subgroups (Fig. 1). Osteocalcin was significantly lower in the severe AIS subgroup than either in the mild and moderate subgroups (Fig. 1A). Interestingly, hormonal activity of the adipose tissue (expressed as leptin level) was significantly higher in the severe AIS girls vs. mild subgroup only (Fig. 1B). Furthermore, adipose tissue distribution (evaluated by W/HtR calculation) was significantly higher in the severe AIS subgroup comparing to the both mild and moderate AIS subjects (Fig. 1C).

Significant correlations between leptin and anthropometrical parameters as BMI z-score and W/HtR were shown. Moreover, leptin level correlated significantly with body composition in the manner as expected (vs. FAT positively and vs. FFM, TBW and PMM negatively) (Table 3.). Osteocalcin level showed significant negative relation to the BMI ($r = -0.238$, $p<0.05$), but after adjustment to age by BMI z-score calculation this correlation was no longer significant. There were no other significant correlations between bone turnover markers vs. neither classical anthropometry and body composition parameters assessed by bioelectrical impedance.

However, there was a significant negative correlation between NTx and leptin level (Fig. 2).

Table 1. Basal characteristics of the total studied AIS population

N	77
Age [years]	14.7 ± 2.17
Corrected height [cm]	166.55 ± 9.48
Corrected height Z score [SD]	0.93 ± 0.99
Weight [kg]	51.28 ± 9.62
BMI [kg/m²]	18.38 ± 2.56
BMI Z score [SD]	-0.70 ± 1.03
W/HtR	0.43 ± 0.04
FAT [%]	21.47 ± 5.56
FFM [%]	77.69 ± 9.03
PMM [%]	74.55 ± 5.73
TBW [%]	56.91 ± 6.34
Cobb's angle [°]	25.21 ± 15.32

Data are expressed as mean ± standard deviation

Abbreviations: BMI – body mass index, FAT – fat mass, FFM – fat free mass, PMM – predicted muscle mass, TBW – total body water, WHR – waist hip ratio, W/HtR – waist height ratio

Table 2. Multivariate regression analysis of the variables influencing the curve magnitude of studied population (adjusted to age and Tanner stage)

Variable	Coefficient β	Cobb's angle	Adjusted R^2
Log Leptin [ng/ml]	0.243	$p < 0.05$	0.062
Log OC [ng/ml]	-0.260	$p < 0.05$	0.082
Log NTx [nM/BCE]	-0.381	$p < 0.01$	0.109
W/HtR	0.314	$p < 0.01$	0.075
FAT [%]	0.268	$p < 0.05$	0.083

Abbreviations: FAT – fat mass, NTx – amino-terminal collagen crosslinks, OC – osteocalcin, W/HtR – waist height ratio

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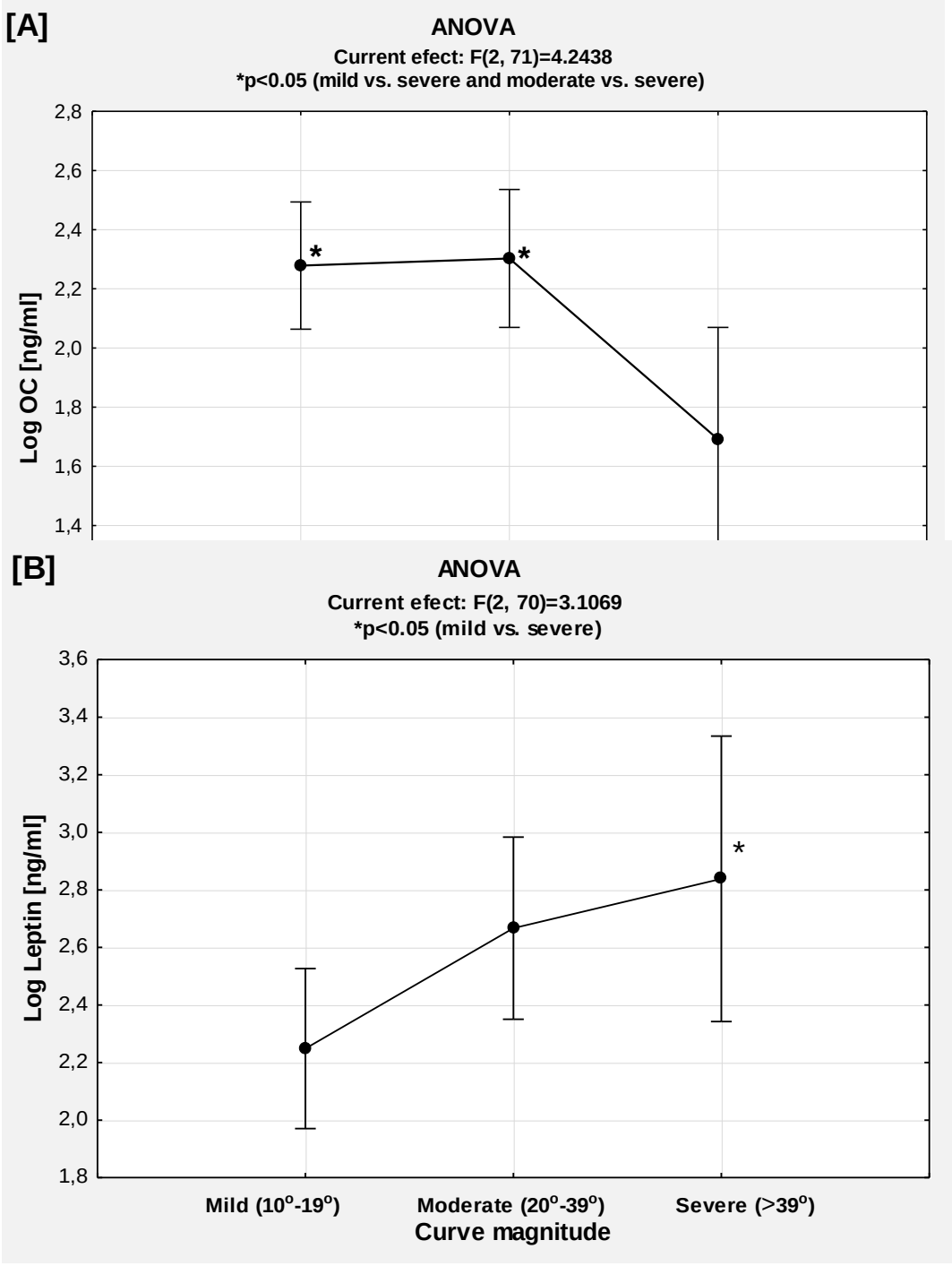
Figure 1. Comparison of osteocalcin (OC) [A], leptin [B] and waist to height ratio (W/HtR) [C] among the scoliotic curve severity subgroups by one-way analysis of variance (ANOVA)



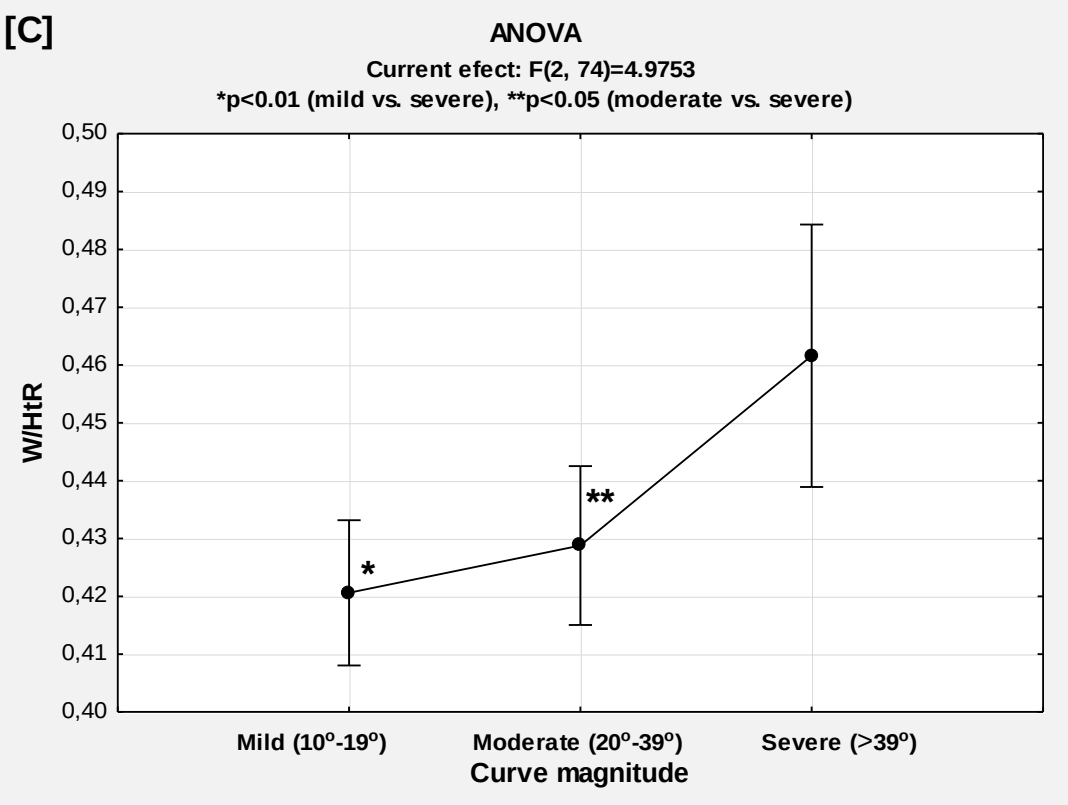


Table 3. Correlations between leptin level and anthropometrical variables in the studied AIS group

	Log Leptin [ng/ml]	
	Pearson's correlation	**Significance**
BMI z-score [SD]	0.372	$p < 0.001$
W/HtR	0.487	$p < 0.0001$
FAT [%]	0.583	$p < 0.000001$
FFM [%]	-0.581	$p < 0.000001$
TBW [%]	-0.581	$p < 0.000001$
PMM[%]	-0.570	$p < 0.000001$

Abbreviations: BMI – body mass index, FAT – fat mass, FFM – fat free mass, PMM – predicted muscle mass, TBW – total body water, W/HtR – waist to height ratio

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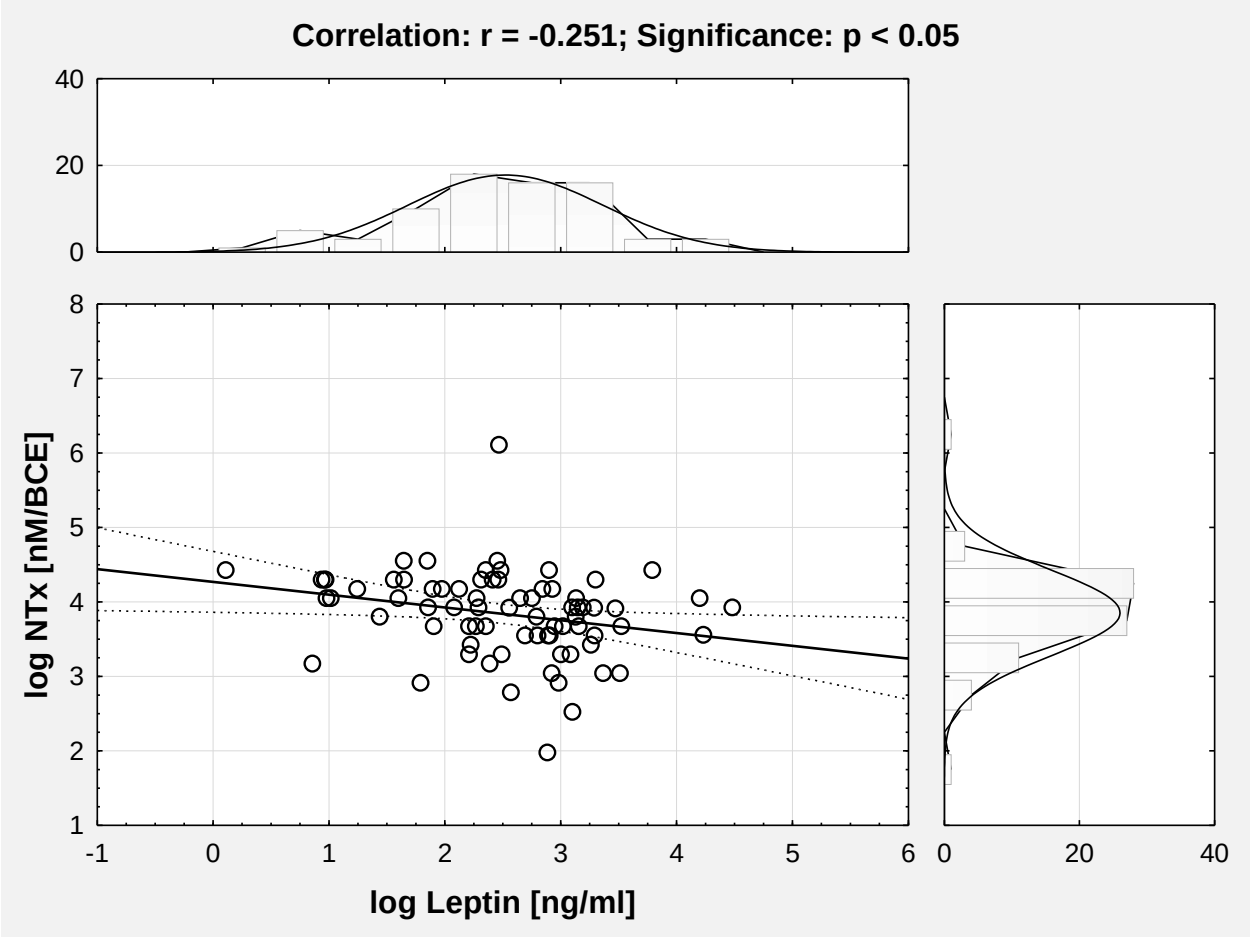
Figure 2. Correlation between amino-terminal collagen crosslinks (log NTx) and leptin (log Leptin) in the AIS girls



4. Discussion

This large scale cross-sectional study confirmed the significant relationship between spinal deformity and bone turnover markers and leptin level in AIS girls. Bone metabolism was significantly more disturbed in severe than in mild and moderately AIS affected patients. Multivariate regression analysis confirmed also our previous study [21], that Cobb's angle was independently associated with variations of the adipose tissue distribution and body composition (W/HtR and FAT) after age and Tanner stage adjustment. Interestingly, spinal deformity correlates with FAT and W/HtR, in the same positive manner as with leptin level, whilst a significant correlation for bone turnover has the opposite negative relation.

Correct body composition, consisting of both adipose tissue and fat free mass, is essential for normal growth and stabilization of the skeletal system, especially the vertebral column. The nutritional status in children is normally assessed by means of height, weight and body mass index (BMI). The obtained results have to be interpreted according to the percentile charts for each parameter. However, this way of anthropometrical analysis does not assess the body composition and adipose tissue distribution. The results of a longitudinal study suggest that changes in BMI

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percentile may not accurately reflect changes in adiposity in children over time, particularly in adolescents and children with lower BMI [22]. The majority of studies performed in scoliotic children indicate that their BMI is lower than in the healthy population [23-25]. Areal BMD (aBMD) measured by dual-energy X-ray absorptiometry (DXA) is currently the gold standard not only for the diagnosis of osteoporosis but also for the body composition evaluation. However, a noninvasive body composition assessment technique is currently available, based on bioelectrical impedance analysis (BIA). A good correlation between BIA and DXA has been reported in estimating adiposity in the different groups of patients [26, 27]. BIA is relatively simple, quick, non-invasive and readily accessible compared to other more sophisticated methods, such as quantitative computed tomography (qCT) or DXA. A more widespread use of DXA in children is limited mainly by its costs and exposure to X-ray radiation. The process of BIA validation resulted in the development of standards and centile charts for healthy children [28]. In the present study body FAT% assessed by BIA correlated significantly with Cobb's angle, and there were also significant relations between all body composition parameters and leptin level. Body composition analysis by BIA has only been used in two studies: our preliminary report which showed a significant correlation between BIA parameters and curve severity, and revealed the potential usefulness of the method in the assessment of IS population [21] and a study by Ramirez et al. [29] in a group of 27 girls with adolescent IS (AIS) surgery candidates, with a large age distribution (13-26 years). Ramirez et al. observed that body composition parameters were significantly lower in the AIS patients than in healthy controls, although this may be attributable to the very high degree of underweight (55.6% with BMI less than 18.5) and the severity of spinal deformity (mean max. Cobb's angle was 66°) . However, the impact of body components on the grade of scoliosis severity was not evaluated in this study [29].

In our study body composition parameters correlated significantly with leptin level. The same results was found in the very recent study conducted in the Chinese AIS population [30]. Study published by Lee et al. revealed that, curve severity was inversely and independently associated with both axial and peripheral bone mineral density (BMD) [18]. These findings may explained our data showing negative correlation between curve magnitude and bone turnover markers.

Currently serum OC levels are used to evaluate bone metabolism, as a bone formation marker. However, recently increasing data have emerged to support extra-skeletal effects of OC [16,17, 31]. In the present study OC showed a significant inverse relationship to the scoliotic curve severity. Similar findings are recently studied by Chen et al. who showed that OC was significantly and negatively related to the Cobb's angle [32]. We also found a significant negative OC correlation vs. BMI, but after age adjustment by the BMI z-score calculation the association was no longer significant. Similar relation between OC and BMI was found by Dubnov-Raz et al. in the group 160 of healthy adolescent girls [33]. The other study showed the inverse significant relation between OC and either adiposity (BMI and fat mass) and leptin level but in the group of adolescent boys [34]. In our study leptin was negatively correlated with NTx in AIS girls. To our knowledge, such relation was not previously described in the literature. We can suspect that altered bone metabolism in the AIS patients seems to be related to the leptin activity pathway.

Abnormal growth pattern is considered an etiological model of IS development, especially during puberty. Nicolopoulos et al. in their study showed, that girls with AIS had significantly different silhouette and its components than controls: taller height, sedentary height and longer lower extremities [5]. Similarly, in a study by Cheung et al. encompassing 598 girls with IS, girls in the

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prepubertal period (Tanner I) were significantly shorter and had shorter sedentary height and between-arms distance compared to the control group; with the progression of puberty (from Tanner II to IV), the height, sedentary height and between-arms distance were significantly greater in girls with IS [35]. However these studies did not compare bone turnover markers and leptin within the IS group in respect of the severity of spinal deformity. In our study, bone turnover markers and leptin were related to the curve severity independently form age and pubertal development (expressed as Tanner stage).

Abnormal pattern of growth is a common finding in children with IS. Leptin, produced mainly by the adipose tissue, is a hormonal factor, which influences both growth and bone mineralization [16, 17, 36]. In the study by Qiu et al., the leptin level was significantly lower in girls with AIS than in controls and correlated with bone mineral density [3]. Other authors emphasize that the role of leptin in the pathogenesis of scoliosis may be associated with the important difference between spine growth velocity compared to the extremities [1, 4, 6, 37] and a lower bioavailability [30, 38] or leptin resistance [39]. Our data confirmed the importance of leptin in the AIS pathogenesis in relation to the bone metabolism.

The waist/height ratio (WHtR) seems to be a useful parameter for anthropometrical evaluation of the severity of deformity in children with IS. In our study, WHtR correlated significantly with spinal deformity and leptin level. WHtR is now widely studied with the aim to find relatively simple parameters of fat tissue distribution in connection with visceral obesity and its comorbidities. A recent analysis showed that WHtR is better than the waist/hip ratio (WHR) for the prognosis of visceral obesity and its comorbidities [40-42]. However, waist circumference measurement may be technically difficult, especially in underweight children with very severe scoliosis in lumbar spine. Therefore, the usefulness of WHtR as a prognostic parameter in idiopathic scoliosis needs to be further investigated.

The major limitations of our study are the lack of the possibility to evaluate the bone mineral density and a relatively low number of the severe curve magnitude patients. Nevertheless, the obtained differences concerning the bone turnover markers and leptin after multifactorial adjustments were statistically significant. Furthermore, to our knowledge, there are no other publications concerning the relationship between bone turnover markers and leptin in AIS patients. Further research in that area of adolescent idiopathic scoliosis is warranted, especially in the subgroup of patient with severe form of AIS.

5. Conclusions

Bone metabolism in AIS girls seems to be altered and significantly related to the scoliotic curve severity. Leptin may be a crucial link in the cross-talk between bone turnover and body composition in this group of patients. The useful tools for anthropometrical analysis in that topic seem to be waist to height ratio (WHtR) and body composition based on bioelectrical impedance analysis (BIA). Further investigations concerning crosstalk between bone and adipose tissue especially in the relation to the curve magnitude in patients with adolescent idiopathic scoliosis are warranted.

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and P.M.; project administration, E.M., and J.D.; funding acquisition, E.M., and J.D. All authors have read and agreed to the published version of the manuscript."

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References

1. Burwell, R.G.; Aujla, R.K.; Grevitt, M.P. et al. Pathogenesis of adolescent idiopathic scoliosis in girls – a double neuro-osseous theory-involving disharmony between two nervous systems, somatic and autonomic expressed in the spine and hormones with implications for medical therapy. *Scoliosis* **2009**, *4*, 29. doi: 10.1186/1748-7161-4-24.
2. Mao, S.; Jiang, J.; Sun, X.; et al. Timing of menarche in Chinese girls with and without adolescent idiopathic scoliosis: current results and review of the literature. *Eur. Spine J.* **2011**, *20*, 260-265. doi: 10.1007/s00586-010-1649-6.
3. Qiu, Y.; Sun, X.; Qiu, X.; et al. Decreased circulating leptin level and its association with body and bone mass in girls with adolescent idiopathic scoliosis. *Spine* **2007**, *32*, 2703-2710.
4. Burwell, R.G.; Aujla, R.K.; Kirby, A.S.; et al. Body mass index of girls in health influences menarche and skeletal maturation: a leptin-sympathetic nervous system focuses on the trunk with hypothalamic asymmetric dysfunction in the pathogenesis of adolescent idiopathic scoliosis? *Stud. Health Technol. Inform.* **2008**, *140*, 9-21. doi: 10.3233/978-1-58603-888-5-9.
5. Nicolopoulos, K.S.; Burwell, R.G.; Webb, J.K. Stature and its components in adolescent idiopathic scoliosis. Cephalo-caudal disproportion in the trunk in girls. *J. Bone Joint Surg. Br.* **1985**, *67*, 594-601.
6. Burwell, R.G.; Dangerfield, P.H.; Moulton, A.; Anderson S.I. Etiologic theories of idiopathic scoliosis: autonomic nervous system and the leptin-sympathetic nervous system concept for the pathogenesis of adolescent idiopathic scoliosis. *Stud. Health Technol. Inform.* **2008**, *140*, 197-207. doi: 10.3233/978-1-58603-888-5-197.
7. Burwell, R.G.; Dangerfield, P.H.; Moulton, A.; Grivas, T.B.; Cheng, J.C.Y. Whither the etiopathogenesis (and scoliogeny) of adolescent idiopathic scoliosis? Incorporating presentations on scoliogeny at the 2012 IRSSD and SRS meetings. *Scoliosis*, **2013**, *8*. doi: 10.1186/1748-7161-8-4.
8. Schlosser, T.P.C.; van der Heijden, G.J.M.G.; Versteeg, A.L.; Castelein, R.M. How 'idiopathic' is adolescent idiopathic scoliosis? A systematic review on associated abnormalities. *PLoS ONE* **2014**, *9*, e97461. doi: 10.1371/journal.pone.0097461.
9. Li, X.F.; Li, H.; Liu, Z.D., Dai, L.Y. Low bone mineral status in adolescent idiopathic scoliosis. *Eur. Spine J.* **2008**, *17*, 1431-1440.
10. Cheung, C.S.; Lee, W.T., Tse, Y.K.; et al. Generalized osteopenia in adolescent idiopathic scoliosis-association with abnormal pubertal growth, bone turnover, and calcium intake? *Spine* **2006**, *31*, 330-338.
11. Lee, W.T.; Cheung, C.S.; Tse, Y.K.; Guo, X.; Qin, L.; Ho S.C.; Lau, J.; Cheng, J.C. Generalized low bone mass of girls with adolescent idiopathic scoliosis is related to inadequate calcium intake and weight bearing physical activity in peripubertal period. *Osteoporos. Int.* **2005**, *16*, 1024-1035.
12. Yu, W.S.; Chan, K.Y.; Yu, F.W.; Yeung, H.Y.; Ng, B.K.; Lee, K.M.; Lam, T.P.; Cheng, J.C. Abnormal bone quality versus low bone mineral density in adolescent idiopathic scoliosis: a case-control study with in vivo high-resolution peripheral quantitative computed tomography. *Spine J.* **2013**, *13*, 1493-1499.
13. Cheuk, K.Y.; Zhu, T.Y.; Yu, F.W.P.; et al. Abnormal bone mechanical and structural properties in adolescent idiopathic scoliosis: a study with finite element analysis and structural model index. *Calcif. Tissue Int.* **2015**, *97*, 343-352. doi: 10.1007/s00223-015-0025-2.
14. Nikolova, S.; Yablanski, V.; Vlaev, E.; et al. In search of biomarkers for idiopathic scoliosis: leptin and BMP4 functional polymorphisms. *J. Biomark.* **2015**, *2015*, Article ID 425310, 5 pages.
15. Schwetz, V.; Pieber, T.; Obermayer-Pietsch, B. The endocrine role of the skeleton: background and clinical evidence. *Eur J. Endocrinol.* **2012**, *166*, 959-967.
16. Reid, I.R. Fat and bone. *Arch. Biochem. Biophys.* **2010**, *503*, 20-27.
17. Confavreux, C.B.; Levine, R.L.; Karsenty, G. A paradigm of integrative physiology, the crosstalk between bone and energy metabolism. *Mol. Cell. Endocrinol.* **2009**, *310*, 21-29.

18. Lee, W.T.K.; Cheung, C.S.K.; Tse, Y.K.; et al. Association of osteopenia with curve severity in adolescent idiopathic scoliosis: a study of 919 girls. *Osteoporos. Int.* **2005**, *16*, 1924-1932.
19. Bjure, J.; Nachemson, A. Non-treated scoliosis. *Clin. Orthop. Relat. Res.* **1973**, *93*, 44-52.
20. de Onis, M.; Onyango, A.W.; Borghi, E.; Siyam, A.; Nishida, C.; Siekmann, J. Development of a WHO growth reference for school-aged children and adolescents. *Bull. World Health Organ.* **2007**, *85*, 660-667.
21. Matusik, E.; Durmala, J.; Matusik, P.; Piotrowski, J. Evaluation of nutritional status of children and adolescents with idiopathic scoliosis – a pilot study. *Ortop. Traumatol. Rehabil.* **2012**, *14*, 351-362.
22. Demerath, E.W.; Schubert, C.M.; Maynard, L.M.; et al. Do changes in body mass index percentile reflect changes in body composition in children? Data from the Fels longitudinal study. *Pediatrics*, **2006**, *117*, 487-495.
23. Wei-Jun, W.; Xu, S.; Zhi-Wei, W.; Xu-Sheng, Q.; Zhen, L.; Yong, Q. Abnormal anthropometric measurements and growth pattern in male adolescent idiopathic scoliosis. *Eur. Spine J.* **2012**, *21*, 77-83.
24. Davey, R.C.; Cochrane, T.; Dangerfield, P.H.; Chockalingam, N.; Dorgan, J.C. *Anthropometry and body composition in females with adolescent idiopathic scoliosis.* In: Sawatzky, B.J. (ed) International Research Society of Spinal Deformities Symposium, University of British Columbia, **2004**, pp. 323-326.
25. Dangerfield, P.H.; Davey, R.C.; Chockalingam, N.; Cochrane, T.; Dorgan, J.C. Body composition in females with adolescent idiopathic scoliosis (AIS) [Abstract]. *J. Bone Joint Surg. Br.* **2005**, *88-B suppl. 2*, 230.
26. de Lorenzo, A.; Sorge, S. P.; Iacopino, L.; Andreoli, A.; de Luca, P.P.; Stasso, G. F. Fat-free mass by bioelectrical impedance vs. dual-energy X-ray absorptiometry (DXA). *Appl. Radiat. Isot.* **1998**, *49*, 739-741.
27. Thomson, R.; Brinkworth, G.D.; Buckley, J.D.; Noakes, M.; Clifton, P.M. Good agreement between bioelectrical impedance and dual-energy X-ray absorptiometry for estimating changes in body composition during weight loss in overweight young women. *Clin. Nutr.* **2007**, *26*, 771-777.
28. McCarthy, H.D.; Cole, T.J.; Fry, T.; Jebb, S.A.; Prentice, A.M. Body fat reference curves for children. *Int. J. Obes.* **2006**, *30*, 598-602.
29. Ramirez, M.; Martinez-Llorens, J.; Sanchez, J.F.; et al. Body composition in adolescent idiopathic scoliosis. *Eur. Spine J.* **2012**, *22*, 324-329. doi: 10.1007/s00586-012-2465-y.
30. Tam, E.M.; Liu, Z.; Lam, T.P.; Ting, T.; Cheung, G.; Ng, BK.; Lee, SK.; Qiu, Y.; Cheng, J.C. Lower muscle mass and body fat in adolescent idiopathic scoliosis are associated with abnormal leptin bioavailability. *Spine* **2016**, *41*, 940-946. doi: 10.1097/BRS.0000000000001376.
31. Neve, A.; Corrado, A.; Cantatore, F.P. Osteocalcin: skeletal and extra-skeletal effects," *J. Cell. Physiol.* **2013**, *228*, 1149-1153.
32. Chen, H.; Zhang, J.; Wang, Y.; Cheuk, K.Y.; Hung, A.L.H.; Lam, T.P.; Qiu, Y.; Feng, Y.Q.; Lee, W.Y.W.; Cheng, J.C.Y. Abnormal lacuno-canalicular network and negative correlation between serum osteocalcin and Cobb angle indicate abnormal osteocyte function in adolescent idiopathic scoliosis. *FASEB J.* **2019**, *33*, 13882–13892.
33. Dubnov-Raz, G.; Ish-Shalom, S.; Chodick, G.; Rozen, G. S.; Giladi, A.; Constantini, N.W. Osteocalcin is independently associated with body mass index in adolescent girls. *Pediatr. Obes.* **2012**, *7*, 313-318.
34. Jurimae, J.; Latt, E.; Maestu, J.; et al. Osteocalcin is inversely associated with adiposity and leptin in adolescent boys," *J. Ped. Endocrinol. Metab.* **2015**, *28*, 571-577. doi: 10.1515/jpem-2014-0432.
35. Cheung, C.S.K.; Lee, W.T.K.; Tse, Y.K.; et al. Abnormal peri-pubertal anthropometric measurements and growth pattern in adolescent idiopathic scoliosis: a study of 598 patients. *Spine*, **2003**, *28*, 2152-2157.
36. Dimitri, P.; Jacques, R.M.; Paggiosi, M.; et al. Leptin may play a role in bone microstructural alterations in obese children. *J Clin. Endocrinol. Metab.* **2015**, *100*, 594-602. doi: 10.1210/jc.2014-3199.
37. Hamrick, M.W.; Pennington, C.; Newton, D.; Xie, D.; Isales, C. Leptin deficiency produces contrasting phenotypes in bones of the limb and spine. *Bone* **2004**, *34*, 376-383.
38. Liu, Z.; Tam, E.M.S.; Sun, G.Q.; et al. Abnormal leptin bioavailability in adolescent idiopathic scoliosis. *Spine* **2012**, *37*, 599-604.
39. Man, G.C.; Tam, E.M.; Wong, Y.S.; Hung, V.W.; Hu, Z.; Lam, T.P.; Liu, Z.; Cheung, W.H.; Ng, T.B.; Zhu, Z.; Qiu, Y.; Cheng, J.C. Abnormal Osteoblastic Response to Leptin in Patients With Adolescent Idiopathic Scoliosis. *Sci Rep.* **2019,** *20*, 17128. doi: 10.1038/s41598-019-53757-3.
40. Mokha, J.S.; Srinivasan, S.R.; DasMahapatra, P.; et al. Utility of waist-to-height ratio in assessing the status of central obesity and related cardiometabolic risk profile among normal weight and

overweight/obese children: The Bogalusa Heart Study. *BMC Pediatrics*, **2010**, *10*, 73-79. doi: 10.1186/1471-2431-10-73.

41. Browning, L.M.; Hsieh, S.D.; Ashwell, M. A systematic review of waist-to-height ratio as a screening tool for the prediction of cardiovascular disease and diabetes: 0-5 could be a suitable global boundary value. *Nutr. Res. Rev.* 2010, 23, 247-269.
42. Yan, W.; Bingxian, H.; Hua, Y.; et al. Waist-to-height ratio is an accurate and easier index for evaluating obesity in children and adolescents. *Obesity*, **2007**, *15*, 748-752.