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Insulin-like growth factor-1 positively associated with bone formation markers and creatine kinase in adults with general physical activity

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Abstract

Background: The Insulin-like growth factor-1 (IGF-1) is primarily synthesized by hepatocytes in a growth hormone (GH)-dependent manner, it is also produced by bone and muscle. The effects of exercise on the associations between IGF-1 levels and bone turnover markers (BTM) were found in the previous studies. However, the associations between the levels of IGF-1 and BTM, liver function tests, and skeletal muscle markers in adults with general physical activity were not clear.

Methods: Ninety-four participants were recruited from healthy survey. Blood samples were collected to analyze the levels of IGF-1, total protein (TP), albumin (Alb), total bilirubin (T-Bil), direct bilirubin (D-Bil), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bone alkaline phosphatase (BALP), lactate dehydrogenase (LDH), creatine kinase (CK), creatinine (CRTN), and glucose. Urine samples were collected to analyze the CRTN and deoxypyridinoline (Dpd) levels.

Results: The positively significant associations were found between the IGF-1 levels and the levels of ALP, BALP, and CK, respectively. No significant associations were found between the IGF-1 levels and the levels of TP, Alb, A/G, T-Bil, D-Bil, AST, ALT, LDH, glucose, urinary CRTN, urinary Dpd, and Dpd/CRTN ratios, respectively.

Conclusion: The serum IGF-1 levels associated with the levels of skeletal muscle and bone formation markers (BFM), not the bone resorption markers under general physical activity in the healthy adults. The physician needs to consider the effects of bone formation and skeletal muscle markers on the IGF-1 levels in the management of IGF-1-related disorders.

KEYWORDS
alkaline phosphatase, bone alkaline phosphatase, bone turnover markers, creatine kinase and bone formation markers, insulin-like growth factor-1

1 | INTRODUCTION

Insulin-like growth factor-I (IGF-1) performs its activity after birth when the liver becomes its main source and under the control of growth hormones (GH), which plays a critical role in all aspects of skeletal development and bone remodeling by promoting the proliferation, differentiation, and function of chondrocytes, osteoblasts, and osteoclasts.[1] There was a strongly negative correlation between plasma IGF-1 levels and hepatic injury and the circulating IGF-1 levels may have predictive value for determining hepatic damage in diabetic rats.[2] Moreover, it is being increasingly noticed that patients with liver disease develop bone loss that can result in fracture and affect the life quality.[3] Langlois et al.[4] showed that the correlation between circulating IGF-1 levels and bone mineral density (BMD) was relatively strong. Many bone turnover markers (BTM) including bone formation and bone resorption involved bone development. Serum bone-specific alkaline phosphatase (BAP), osteocalcin (OC) and C-terminal or N-terminal propeptides of type I procollagen (PICP; PINP) were bone formation markers, however, urinary deoxypyridinoline (Dpd), serum carboxy- and amino-terminally cross-linked telopeptides of type I collagen (CTX and NTX) and Tartrate-resistant acid phosphatase 5b (TRAP5b) were regarded as bone resorption markers in clinical performance.[5] Recently, the interactions between bone and skeletal muscles via local and humoral signaling pathways in addition to their musculoskeletal function were found, even if the mechanisms related to the interactions between skeletal muscles and bones remain unclear.[6]

Low-circulating IGF-1 levels were associated with hepatic steatosis, and the association in patients with increased alanine aminotransferase (ALT) levels was stronger than that in patients without increased ALT levels.[7] After participating in an eight-week high-force eccentric-cycle ergometry program, the subjects had higher serum creatine kinase (CK) levels and muscle IGF-1Ea (a muscle-specific isoform of IGF-1) than those before.[8] In recent study,[9] the circulating IGF-1 levels were significantly higher in the obese patients than those in the controls, and the significant association of BAP levels with IGF-1 levels was found in the obese patients.

The positive associations between the IGF-1 and the risks of breast cancer, papillary thyroid cancer, and prostate cancer were found in the previous studies,[10-13] but the risk of gastric cancer decreased in the individuals with IGF-1 gene rs2195239 polymorphism.[14] Nevertheless, no significant difference of IGF-1 levels between the breast cancer patients and the controls was found in our previous study[15] and another studies.[16,17] In addition to the influences of menopausal statuses,[10] racial variations,[18] gene polymorphism,[14] and subtypes of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor 2 (HER2),[19] the associations between the IGF-1 levels and biomarkers of skeletal muscles, liver function and bone modulation were worthy to investigate. Above-mentioned studies, the associations between the IGF-1 levels and biomarkers of skeletal muscles, liver function and bone modulation were shown in the participants with exercise programs or the patients with hepatic and metabolic diseases. In this study, the associations among them were investigated in the healthy adults with general physical activity. The associations of the biomarkers of skeletal muscles, liver function, and bone modulation with the circulating IGF-1 levels in the management of IGF-1-related disorders need to be evaluated.

2 | MATERIALS AND METHODS

2.1 | Subjects

Ninety-four participants including 35 males and 59 females (ages: 21–73 years, mean: 44.6 years) were recruited from healthy survey of a Kaohsiung Medical University Hospital. Those with the cancer histories, diabetes mellitus, hypertension, immunodeficiency disease, or inflammatory signs were excluded from the present study. This study was approved by the Ethics Committee of the Kaohsiung Medical University Hospital (KMUH-IRB-940225), and the informed consent of the subjects was obtained.

2.2 | Sample collections and analysis

Blood samples were collected from all participants after an 8-hour fasting period in plain tubes and centrifuged at 2500 rpm for 10 minutes as soon as they had been collected. Prolonged exposure of the samples to light, especially direct sunlight, was avoided. The serum was separated immediately and stored at −20℃ until used for assay. Stored serum samples were assayed within one week after sampling. Urine samples were collected between 9 and 11 am. Borate (1 g/L) was added to urine samples to prevent bacterial growth. The urine samples were stored at −20°C until analyzed.

Urine creatinine (CRTN) levels and serum total protein (TP), albumin (Alb), total bilirubin (T-Bil), direct bilirubin (D-Bil), aspartate aminotransferase (AST), ALT, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), CK, CRTN, and glucose levels were measured by the Beckman Synchron CX-7 analyzer (Beckman Coulter Inc., Fullerbon, CA, USA). The TP and Alb levels were analyzed by the Biuret and bromocresel green (BCG) methods, respectively. The T-Bil and D-Bil levels were analyzed by the Diazo method. The AST, ALT, ALP, CK, and LDH levels were analyzed by the UV-kinetic method. The urine and serum CRTN levels were analyzed by the Jaffe's reaction. The glucose levels were analyzed by the HK/G6PD method.

Serum BALP level was measured by the Access Ostase assay (immunoassay method) by the Access II analyzer (Beckman Coulter Inc., Fullerbon, CA, USA).

Serum IGF-1 level was determined by the solid-phase, enzyme-labeled chemiluminescent immunometric assay by DPC's IMMULITE 2000 (Diagnostic Products Corporation, CA, USA).

Urinary Dpd levels were measured by enzyme immunoassay on microtiter plates using reagents (Pyrilin R5 TM-D) supplied by the manufacturer (Metra Biosystems, France).

2.3 | *Statistical analysis*

Data were expressed as mean ±standard deviation (SD). The associations between IGF-1 levels and the levels of TP, Alb, A/G, T-Bil, D-Bil, I-Bil, AST, ALT, ALP, BALP, LDH, CK, glucose, urinary CRTN, urinary Dpd, and Dpd/CRTN ratio were performed by using simple regression. The alpha-level 0.05 was used to distinguish the difference in statistical significance.

3 | RESULTS

The IGF-1 levels and the levels of TP, Alb, A/G, T-Bil, D-Bil, I-Bil, AST, ALT, ALP, BALP, LDH, CK, glucose, urinary CRTN, urinary Dpd, and Dpd/CRTN ratios were shown in the Table 1. As shown in the Table 1 and Figure 1, the positively significant associations were found between the IGF-1 levels and the levels of ALP, BALP and CK, respectively (p=0.0071, 0.00032 and 0.023, respectively). Moreover, the association between the levels of BALP and IGF-1 was stronger than that between the serum IGF-1 and ALP levels. The associations between the IGF-1 levels and the levels of TP, Alb, A/G, T-Bil, D-Bil, I-Bil, AST, ALT, LDH, glucose, urinary CRTN, urinary Dpd, and Dpd/CRTN ratio were not significant (p>0.05).

TABLE 1 Correlations between the IGF-1 levels and the levels of TP, Alb, A/G, T-Bil, D-Bil, I-Bil, AST, ALT, ALP, BALP, LDH, CK, Glucose, urinary CRTN and urinary Dpd and Dpd/CRTN ratios in the healthy subjects

Parameters	subjects (N=94)	r	p-values*
IGF−1 (ng/mL)	256.2±142.7		
TP (gm/dL)	7.17±0.4	0.127	0.224
Alb(gm/dL)	4.13±0.3	0.134	0.196
A/G	1.37±0.2	0.053	0.612
T-Bil (mg/dL)	0.89±0.33	0.058	0.580
D-Bil (mg/dL)	0.11±0.06	−0.0005	0.996
AST (IU/L)	26.55±13.8	−0.128	0.218
ALT (IU/L)	31.13±42.26	−0.05	0.629
ALP (IU/L)	60.4±25.36	0.276	0.0071
B-ALP (ug/L)	12.59±8.95	0.363	0.00032
LDH (IU/L)	149.55±27.76	0.023	0.825
CK (IU/L)	132.86±102.37	0.235	0.023
Glucose (mg/dL)	93.29±12.96	−0.034	0.745
Urinary CRTN (mM)	12.92±7.53	0.161	0.121
Urinary Dpd (nM)	6.52±2.1	0.190	0.067
Dpd/CRTN (x10^{-6})	6.52±2.11	0.082	0.42958

Results were expressed as mean ±SD

*P-values represented the associations between the IGF-1 levels and the levels of TP, Alb, A/G, T-Bil, D-Bil, AST, ALT, ALP, BALP, LDH, CK, Glucose, urinary CRTN and urinary Dpd and Dpd/CRTN ratios, respectively.

4 | DISCUSSION

Several factors affect the interactions between skeletal muscle and bone, the factors include genetic factors, aging, circadian rhythm, nutrition, mechanical factors, and endocrine factors such as IGF-1.[20-22] The IGF-1 is primarily produced by the liver in a GH-dependent manner, but many of the tissues such as bone and skeletal muscle make their own IGF-1 by autocrine and paracrine signaling.[6,22] Exercise-induced mechanical loading may positively influence bone through the stimulation of IGF-1 secretion from skeletal muscle, in spite of the roles of GH/IGF-1 axis in the interactions between skeletal muscle and bone are not fully understood.[6] A 3-year follow-up study demonstrated that IGF-1 levels significantly positively associated with the BMD at both baseline and follow-up.[23] Recently, the review articles revealed that the BMD and bone elongation increased by the actions of IGF-1 on skeletal acquisition through stimulation of extracellular matrix production and growth plate through regulation of cell proliferation and differentiation.[24,25] In this study, there was positively significant correlation between the serum IGF-1 and ALP levels in healthy population, but negative correlation was found between serum IGF-1 and AST levels even though the correlation was not statistical significance. It was consistent to the study of Su et al.[26] that serum IGF-1 levels were positively correlated with serum ALP levels, but were inversely correlated with serum AST levels in the healthy subjects. In additional, the serum IGF-1 level was not significantly correlated with the serum levels of TP, Alb, T-Bil, D-Bil and ALT in the present study. We infer that the interactions between the serum levels of IGF-1 and liver function tests were not found in the healthy population.

The increased serum IGF-1 levels were observed in elite athletes immediately after a maximum exercise test.[27] It may be due to the muscle damaging exercise result in local up-regulation of IGF-1.[22,28] IGF-1 plays an important role in myoblast proliferation, differentiation, and fiber formation after skeletal muscle damage.[22,28,29] Therefore, muscle CK efflux and serum CK levels significantly increased after exercise, but depended on the type of exercise.[30,31] In this study, the positive and significant correlation between the IGF-1 and CK levels was also found in adults with general physical activity.

IGF-1 actions are regulated by IGF-binding proteins (IGFBPs), which are secreted from muscle and may act on bone.[6] Moreover, Tsuchiya et al.[31] suggested that high-force eccentric exercise-induce IGF-1 may activate OC and NTX in acute response. Although the different types of exercise resulted in the various effects of exercise on the IGF-1 levels and bone markers,[27,28,31] the effects of exercise on the associations between IGF-1 levels and BTM were found in the previous studies.[27,28,31-33] However, most of the patients who received blood sampling for clinical diagnosis or monitor were under general physical activity. Therefore, the strong positive associations between the IGF-1 levels and the bone formation marker PINP or the bone resorption marker CTX in young men and women (<55 years old) were shown in the general adult population.[34] However, the IGF-1 levels were positive and significant association with the serum levels of PINP and BALP, not
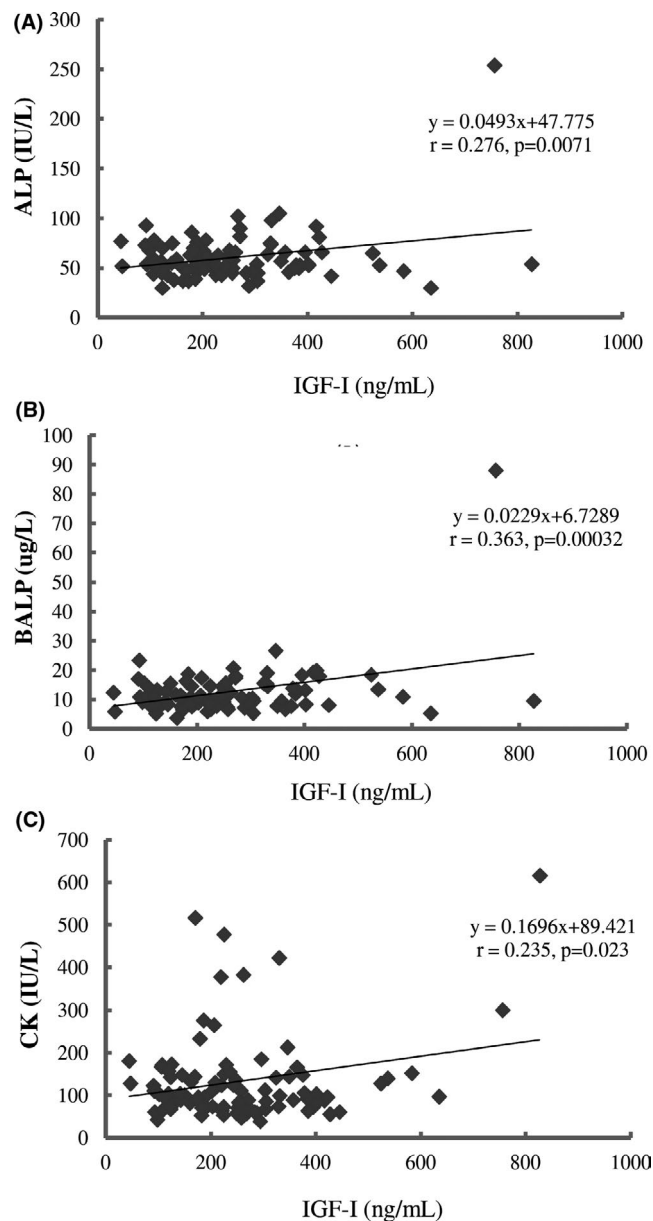
FIGURE 1 Positive associations of ALP levels (A), BALP levels (B), CK levels (C) with IGF-1 levels in the healthy adults with general physical activity

with the serum CTX levels in premenopausal women were shown in the previous study.[35] Likewise, circulating IGF-1 was associated with bone formation markers in postmenopausal women, but not with CTX levels.[36] IGF-1 enhances bone collagen and matrix synthesis and stimulates the replication of cells from the osteoblast lineage.[37] The results of the previous studies[35,36] were consistent to our results, which the IGF-1 levels were positive and significant correlations with ALP and BALP levels, however, the urinary Dpd levels and Dpd/CRTN ratios were not significantly correlated with serum IGF-1 levels without restriction of ages. In the previous study[38] demonstrated that the urinary Dpd/CRTN ratio was found as a useful marker for bone metastasis caused by breast cancer and Dpd can be useful in evaluating therapeutic response. However,

the serum IGF-1 levels associated with bone formation markers, not bone resorption marker under general physical activity in the healthy adults in this study. In our knowledge, the associations between the serum IGF-1 levels and urinary Dpd levels or Dpd/CRTN ratios were not shown in the previous study.

In conclusion, although the IGF-1 is primarily synthesized by hepatocytes in a GH-dependent manner, the serum IGF-1 levels were not associated with the serum levels of liver function tests in the healthy adults. In additional, the IGF-1 is also produced by the bone and muscle and the interactions between bone and muscle were shown in the previous study.[6] In this study, the IGF-1 levels were positive and significant correlations with CK, ALP and BALP levels, but not with the urinary Dpd levels and Dpd/CRTN ratios. Therefore, the serum IGF-1 levels associated with the bone formation markers, not the bone resorption markers under general physical activity in the healthy adults. Recently, IGF-1 is a potential treatment in the patients with muscle atrophy.[29] In clinical performance, the Duchenne muscular dystrophy patients received 6 months of recombinant human insulin-like growth factor-1 therapy improved linear growth.[39] The physician needs to consider the effects of bone formation and muscle damage markers on the IGF-1 levels in the management of IGF-1-related disorders. The less sample size was our limitation. It is worthy to investigate the further studies by a large sample size in the future.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

1. Bikle DD, Wang Y. Insulin-like growth factor-I and bone. *IBMS BoneKEy.* 2011;8(7):328–341. http://dx.doi.org/10.1138/20110521
2. Aksu I, Baykara B, Kiray M, et al. Serum IGF-1 levels correlate negatively to liver damage in diabetic rats. *Biotech Histochem.* 2013;88(3–4):194-201.
3. Nakchbandi IA. Osteoporosis and fractures in liver disease: relevance, pathogenesis and therapeutic implications. *World J Gastroenterol.* 2014;20(28):9427-9438.
4. Langlois JA, Rosen CJ, Visser M, et al. Association between insulin-like growth factor I and bone mineral density in older women and men: the Framingham Heart Study. *J Clin Endocrinol Metab.* 1998;83(12):4257–4262. http://dx.doi.org/10.1210/jcem.83.12.5308
5. Jung K, Lein M. Bone turnover markers in serum and urine as diagnostic, prognostic and monitoring biomarkers of bone metastasis. *Biochim Biophys Acta.* 2014;1846(2):425–438. http://dx.doi.org/10.1016/j.bbcan.2014.09.001
6. Kawao N, Kaji H. Interactions Between Muscle Tissues and Bone Metabolism. *J Cell Biochem.* 2015;116(5):687–695. http://dx.doi.org/10.1002/jcb.25040
7. Völzke H, Nauck M, Rettig R, et al. Association between hepatic steatosis and serum IGF1 and IGFBP-3 levels in a population-based sample. *Eur J Endocrinol.* 2009;161(5):705–713. http://dx.doi.org/10.1530/eje-09-0374

8. Flann KL, LaStayo PC, McClain DA, Hazel M, Lindstedt SL. Muscle damage and muscle remodeling: no pain, no gain? *J Exp Biol*. 2011;214(Pt 4):674-679.

9. Gajewska J, Klemarczyk W, Ambroszkiewicz J, Szamotulska K, Chełchowska M, Weker H. Associations between IGF-1, IGF-binding proteins and bone turnover markers in prepubertal obese children. *J Pediatr Endocrinol Metab*. 2015;28(5–6):563-569.

10. Key TJ, Appleby PN, Reeves GK, Roddam AW. Insulin-like growth factor 1 (IGF1), IGF binding protein 3 (IGFBP3), and breast cancer risk: pooled individual data analysis of 17 prospective studies. *Lancet Oncol*. 2010;11(6):530–542. http://dx.doi.org/10.1016/s1470-2045(10)70095-4

11. Chong YM, Subramanian A, Sharma AK, Mokbel K. The potential clinical applications of insulin-like growth factor-1 ligand in human breast cancer. *Anticancer Res*. 2007;27(3B):1617-1624.

12. Yang L, Tan Z, Li Y, et al. Insulin-like growth factor 1 promotes proliferation and invasion of papillary thyroid cancer through the STAT3 pathway. *J Clin Lab Anal*. 2020;34(12):e23531. http://dx.doi.org/10.1002/jcla.23531

13. Watts EL, Fensom GK, Smith Byrne K, et al. Circulating insulin-like growth factor-I, total and free testosterone concentrations and prostate cancer risk in 200 000 men in UK Biobank. *Int J Cancer*. 2021;148(9):2274–2288. http://dx.doi.org/10.1002/ijc.33416

14. Wang P, Zhou X. Association of IGF-1 gene rs2195239 polymorphism with the risk and clinical features of gastric cancer in a Chinese Han population. *J Clin Lab Anal*. 2020;34(7):e23284. http://dx.doi.org/10.1002/jcla.23284

15. Lee SC, Tsai SM, Hou MF, et al. Increased Igf-I/Igfbp-3 Ratios in Postmenopausal Taiwanese with Breast Cancer, Irrespective of Er and Pr Statuses and Her2 Expression in a Case-Control Study. *J Clin Lab Anal*. 2016;30(1):58–64. http://dx.doi.org/10.1002/jcla.21815

16. Trinconi AF, Filassi JR, Soares JM Jr, Baracat EC. Evaluation of the insulin-like growth factors (IGF) IGF-1 and IGF binding protein 3 in patients at high risk for breast cancer. *Fertil Steril*. 2011;95(8):2753-2755.

17. Eng-Wong J, Perkins SN, Bondy M, et al. Premenopausal breast cancer: estrogen receptor status and insulin-like growth factor-I (IGF-I), insulin-like growth factor binding protein-3 (IGFBP-3), and leptin. *Breast J*. 2009;15(4):426–428. http://dx.doi.org/10.1111/j.1524-4741.2009.00753.x

18. Fowke JH, Matthews CE, Yu H, et al. Racial differences in the association between body mass index and serum IGF1, IGF2, and IGFBP3. *Endocr Relat Cancer*. 2010;17(1):51–60. http://dx.doi.org/10.1677/erc-09-0023

19. Song Q, Huang R, Li J, et al. The diverse distribution of risk factors between breast cancer subtypes of ER, PR and HER2: a 10-year retrospective multi-center study in China. *PLoS One*. 2013;8(8):e72175.

20. Brotto M, Bonewald L. Bone and muscle: interactions beyond mechanical. *Bone*. 2015;80:109-114.

21. Li G, Zhang L, Wang D, et al. Muscle-bone crosstalk and potential therapies for sarco-osteoporosis. *J Cell Biochem*. 2019;120(9):14262–14273. http://dx.doi.org/10.1002/jcb.28946

22. Bikle DD, Tahimic C, Chang W, et al. Role of IGF-I signaling in muscle bone interactions. *Bone*. 2015;80:79–88. http://dx.doi.org/10.1016/j.bone.2015.04.036

23. Kouda K, Iki M, Ohara K, et al. Associations between serum levels of insulin-like growth factor-I and bone mineral acquisition in pubertal children: a 3-year follow-up study in Hamamatsu, Japan. *J Physiol Anthropol*. 2019;38(1):16. http://dx.doi.org/10.1186/s40101-019-0210-5

24. Dixit M, Poudel SB, Yakar S. Effects of GH/IGF axis on bone and cartilage. *Mol Cell Endocrinol*. 2021;519:111052. http://dx.doi.org/10.1016/j.mce.2020.111052

25. Racine HL, Serrat MA. The actions of IGF-1 in the growth plate and its role in postnatal bone elongation. *Curr Osteoporos Rep*. 2020;18(3):210-227.

26. Su WW, Lee KT, Yeh YT, et al. Association of circulating insulin-like growth factor 1 with hepatocellular carcinoma: one cross-sectional correlation study. *J Clin Lab Anal*. 2010;24(3):195–200. http://dx.doi.org/10.1002/jcla.20320

27. Ehrnborg C, Lange KHW, Dall R, et al. The growth hormone/insulin-like growth factor-I axis hormones and bone markers in elite athletes in response to a maximum exercise test. *J Clin Endocrinol Metab*. 2003;88(1):394-401.

28. Eliakim A, Nemet D, Most G, et al. Effect of Gender on the GH-IGF-I Response to Anaerobic Exercise in Young Adults. *J Strength Cond Res*. 2014;28(12):3411–3415. http://dx.doi.org/10.1519/jsc.0000000000000605

29. Ahmad SS, Ahmad K, Lee EJ, et al. Implications of Insulin-Like Growth Factor-1 in Skeletal Muscle and Various Diseases. *Cells*. 2020;9(8):1773. http://dx.doi.org/10.3390/cells9081773

30. Baltusnikas J, Venckunas T, Kilikevicius A, Fokin A, Ratkevicius A. Efflux of creatine kinase from isolated soleus muscle depends on age, sex and type of exercise in mice. *J Sports Sci Med*. 2015;14(2):379-385.

31. Tsuchiya Y, Sakuraba K, Ochi E. High force eccentric exercise enhances serum tartrate-resistant acid phosphatase-5b and osteocalcin. *J Musculoskelet Neuronal Interact*. 2014;14(1):50-57.

32. Rogers RS, Dawson AW, Wang Z, Thyfault JP, Hinton PS. Acute response of plasma markers of bone turnover to a single bout of resistance training or plyometrics. *J Appl Physiol*. 2011;111(5):1353–1360. http://dx.doi.org/10.1152/japplphysiol.00333.2011

33. Barrack MT, Van Loan MD, Rauh MJ, Nichols JF. Physiologic and behavioral indicators of energy deficiency in female adolescent runners with elevated bone turnover. *Am J Clin Nutr*. 2010;92(3):652–659. http://dx.doi.org/10.3945/ajcn.2009.28926

34. Niemann I, Hannemann A, Nauck M, et al. The association between insulin-like growth factor I and bone turnover markers in the general adult population. *Bone*. 2013;56(1):184-190.

35. Adami S, Zivelonghi A, Braga V, et al. Insulin-like growth factor-1 is associated with bone formation markers, PTH and bone mineral density in healthy premenopausal women. *Bone*. 2010;46(1):244-247.

36. Nabipour I, Larijani B, Beigi S, et al. Relationship among insulin like growth factor I concentrations, bone mineral density, and biochemical markers of bone turnover in postmenopausal women. *Menopause*. 2008;15(5):934–939. http://dx.doi.org/10.1097/gme.0b013e31816665a7

37. Guerra-Menéndez L, Sádaba MC, Puche JE, et al. IGF-I increases markers of osteoblastic activity and reduces bone resorption via osteoprotegerin and RANK-ligand. *J Transl Med*. 2013;11(1):271. http://dx.doi.org/10.1186/1479-5876-11-271

38. Hou MF, Lin SB, Yuan SS, et al. Diagnostic value of urine deoxypyridinoline for detecting bone metastases in breast cancer patients. *Ann Clin Lab Sci*. 2003;33(1):55-61.

39. Rutter MM, Wong BL, Collins JJ, et al. Recombinant human insulin-like growth factor-1 therapy for 6 months improves growth but not motor function in boys with Duchenne muscular dystrophy. *Muscle Nerve*. 2020;61(5):623–631. http://dx.doi.org/10.1002/mus.26846