

Increased Plasma Levels of Growth Hormone, Insulin-Like Growth Factor (IGF)-I and IGF-Binding Protein 3 in Pregnant Rats with Exercise

SEBAHAT TURGUT, BÜNYAMIN KAPTANOĞLU,¹ GÜLTEN EMMUNGIL and GÜNFER TURGUT

Departments of Physiology and ¹Biochemistry, Faculty of Medicine, Pamukkale University, Denizli, Turkey

TURGUT, S, KAPTANOĞLU, B., EMMUNGIL, G. and TURGUT, G. *Increased Plasma Levels of Growth Hormone, Insulin-Like Growth Factor (IGF)-I and IGF-Binding Protein 3 in Pregnant Rats with Exercise.* Tohoku J. Exp. Med., 2006, **208** (1), 75-81 — Growth hormone (GH) and insulin-like growth factor-I (IGF-I) are closely related molecules. Insulin-like growth factor-binding protein-3 (IGFBP-3) is a main molecule that binds IGF-I. GH, IGF-I and IGFBP-3 have important roles in growth and development. In this study, we investigated the effects of exercise during pregnancy on maternal plasma levels of GH, IGF-I and IGFBP-3 and on fetal development. We also recorded the weights of placenta, lengths of umbilical cord, fetal body weights, fetal heights, and weights of fetal tissues. Pregnant Wistar Albino rats were divided into two groups: exercise and control groups ($n = 7$ for each). A treadmill exercise was performed as 20 m/min for 20 min/day, once per day for 19 days in exercise group. Blood samples were collected from pregnant rats on 0, 7th, 14th and 20th days of gestation (D) under anesthesia with intracardiac puncture, and maternal plasma levels of GH, IGF-I and IGFBP-3 were determined. Fetuses were taken with cesarean section on D20, and various parameters for fetal growth were measured. Plasma GH and IGF-I levels were elevated in exercising pregnant rats on D14 and D20, respectively, when compared to controls, and IGFBP-3 levels were increased on D14 and D20. Among the growth parameters examined, only fetal body weights and weights of fetal liver were significantly decreased in the exercise group ($p < 0.01$ and $p < 0.05$, respectively). These results indicate that maternal exercise significantly increases plasma levels of GH, IGF-I and IGFBP-3 in the late period of pregnancy but causes adverse effects on fetal growth. — pregnancy; growth hormone; insulin-like growth factor I (IGF-I); insulin-like growth factor binding protein-3 (IGFBP-3); fetus
© 2006 Tohoku University Medical Press

Insulin-like growth factor-I (IGF-I) is a 70-amino-acid single chain polypeptide hormone with similar structural features to insulin (Isaksson et al. 1991). IGF-I plays an important role in the

growth hormone (GH)/insulin-like growth factor pathway (Well 1998; Rosen and Pollak 1999). This pathway is influenced by physical exercise (Jenkins 1999).

Received August 17, 2005; revision accepted for publication October 22, 2005.

Correspondence: Dr. Sebahat Turgut, Pamukkale Üniversitesi Tıp Fakültesi Fizyoloji A.D., Kınıklı/Denizli, Turkey.

e-mail: sturgut@pamukkale.edu.tr

Insulin-like growth factor-binding protein-3 (IGFBP-3) is a 264-amino-acid glycoprotein synthesized in the liver and in other tissues (Ballard et al. 1989). It is the major form of binding proteins in human circulation and function as a major carrier for IGF-I, whose actions may be inhibited and/or enhanced (Rajaram et al. 1997). This protein protects IGF-I from degradation and lowers the free concentration of IGF-I (Collett-Solberg and Cohen 1996).

IGF-I is thought to play an important role in perinatal growth although physiological factors that regulate perinatal growth are not well known. It is believed that IGF-I is a major determinant of fetal growth and GH is likely to be mediated in part via the IGF system (Ogilvy-Stuart et al. 1998).

The increase in plasma GH after exercise has been well documented (Galbo 1985; Flanagan et al. 1997). Plasma IGF-I response to physical exercise has not been demonstrated consistently, some research groups have observed increments, while others showed no changes (Di Luigi et al. 1997; Nguyen et al. 1998; Wallace et al. 1999). IGFBP-3 has the ability to interact with cell surface receptors and increase the half life of IGF-I, thereby potentiating its biological effects (Koistinen et al. 1996). However, attempts to assess possible changes in serum IGFBP-3 concentration in relation to physical exercise have yielded controversial results (Cohich and Clemmons 1993; Schwarz et al. 1996).

Conflicting results have been reported concerning the effect of endurance exercise before or during pregnancy on fetal development. Studies on rats and mice have indicated that endurance exercise during pregnancy causes a decrease in fetal size (Ribeiro et al. 1991; Riemann and Kanstrup Hansen 2000). However, other investigators did not find a correlation between maternal endurance exercise and fetal birth weight (Bell and Palma 2000). We therefore investigated the effects of exercise during pregnancy on the maternal blood levels of GH, IGF-I and IGFBP-3 and fetal development in rats.

MATERIALS AND METHODS

Animals

Adult female Wistar Albino rats were supplied from the Animal Care Unit within Pamukkale University Animal Research Center, Turkey. They were reared under the supervision of a veterinarian, kept in a well-ventilated, noiseless environment and allowed free access to food and water. The female animals were selected randomly and divided into two groups. The female rats were mated with male rats. Pregnancy was approved by vaginal smear. The day which pregnancy was diagnosed was accepted as D0 (1st day). They were grouped as control and exercise group (7 rats in each group).

Animal care and all experimental procedures used were in accordance with those detailed in the Guide for Care and Use of Laboratory Animals, which was published by the U.S. Department of Health and Human Services. The study was approved by Pamukkale University Ethics Committee.

Exercise protocol

Exercise was performed on a motor-driven treadmill (MAY-TME 9805, Commat, Ankara, Turkey). The training programme consisted of running at 20 m/min for 20 min/day, once per day for 19 days for exercise group. Animals were familiarized to the treadmill and run for < 10 min/day during 3 days until the initiation of the training protocol. The speed of the treadmill was gradually increased until the animals were running at the designated speed. Mild electric shocks were used to persuade the animals to run, however, most animals ran voluntarily during their initial run.

Biological parameters

During pregnancy of 21 days, 1 ml of blood was drawn from each animal on the D0, D7 (7th day), D14 (14th day) and D20 (20th day) of gestation. Blood was collected in heparinized tubes. After centrifugation, plasma was stored at -20°C until analysis.

Samples collected on the D0, D7, D14 and D20 of gestation were analyzed for the determination of plasma GH, IGF-I and IGFBP-3 levels with commercial kits using chemiluminescence method (Diagnostic Product Corporation, Los Angeles, CA, USA) by an autoanalyzer (Immulate One, Los Angeles, CA, USA).

On the D20, rats were anesthetized with ketamine (50 mg/kg, Parke Davis, Turkey) and xylazine (5 mg/kg, Alfasan, Turkey). Rats were sacrificed under anesthesia and the abdominal cavity was quickly opened and the

fetus excised. Placenta and fetal weights were measured. Livers, kidneys and hearts of the fetuses were harvested. Weights of the organs were also recorded.

Statistical analysis

Comparisons of results were made with Mann-Whitney's U-test. Wilcoxon test was used for the comparisons within groups. For the multiple comparisons within groups *t*-test was used for the evaluation of paired samples. For all of statistical analyses the SPSS 10.0 packet program were used. Data were expressed as mean \pm s.d. Statistical significance was defined as $p < 0.05$.

RESULTS

Plasma levels of GH, IGF-I and IGFBP-3, fetus numbers, heights and weights of fetuses, weights of fetal liver, heart and kidney, lengths of umbilical cords and placenta weights of pregnant rats having exercise were compared with those of

control pregnant rats. There was no difference in the body weights of rats between control and exercise group at D20. No significant difference was observed in the fetus numbers and heights, fetal heart and kidney weights, lengths of umbilical cord, and placenta weights between the two groups (Table 1). Fetal weights of exercise and control groups were found to be 4.22 ± 0.42 g and 4.78 ± 0.24 g, respectively. There was a decrease of 0.56 g in the fetal weights of exercise group, which was statistically significant ($p < 0.01$, Table 1). In addition, mean weights of fetal liver of exercise group were approximately 66.44 mg and it was significantly lower than those of controls ($p < 0.05$, Table 1).

Plasma levels of GH, IGF-I and IGFBP-3 of pregnant rats obtained on the D0, D7, D14 and D20 of gestation were compared with control rats. GH levels of controls and exercise group on the

TABLE 1. Comparison of parameters measured in exercise and control groups (mean \pm s.d.)

| | Control group (n = 7) | Exercise group (n = 7) |
|----------------------------|-----------------------|------------------------|
| Number of fetuses | 9.13 \pm 2.03 | 9.00 \pm 1.26 |
| Fetus weight (g) | 4.78 \pm 0.24 | 4.22 \pm 0.42** |
| Fetus height (mm) | 51.81 \pm 1.18 | 51.89 \pm 0.61 |
| Placenta weight (g) | 0.44 \pm 0.03 | 0.48 \pm 0.06 |
| Umbilical cord length (mm) | 27.94 \pm 1.20 | 28.04 \pm 1.18 |
| Fetal liver weight (mg) | 270.77 \pm 34.55 | 204.33 \pm 54.12* |
| Fetal kidney weight (mg) | 19.38 \pm 1.6 | 17.59 \pm 2.8 |
| Fetal heart weight (mg) | 23.70 \pm 2.5 | 25.91 \pm 4.19 |

* $p < 0.05$, ** $p < 0.01$.

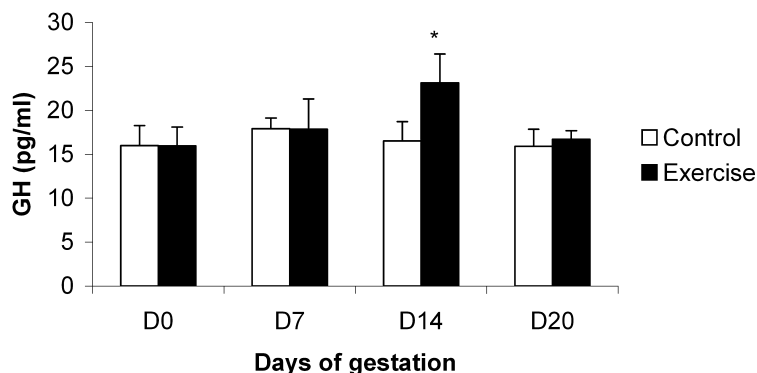


Fig. 1. Changes in the plasma levels of GH during pregnancy in controls and exercise group.
* $p < 0.05$.

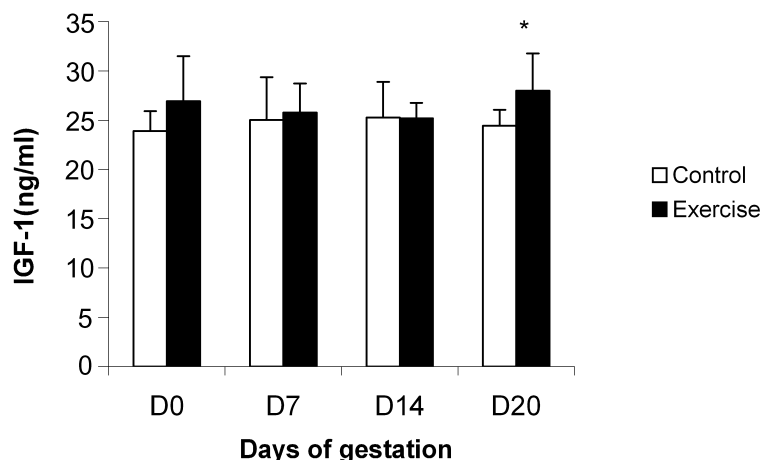


Fig. 2. Changes in the plasma levels of insulin-like growth factor I (IGF-I) during pregnancy in controls and exercise group.
* $p < 0.05$.

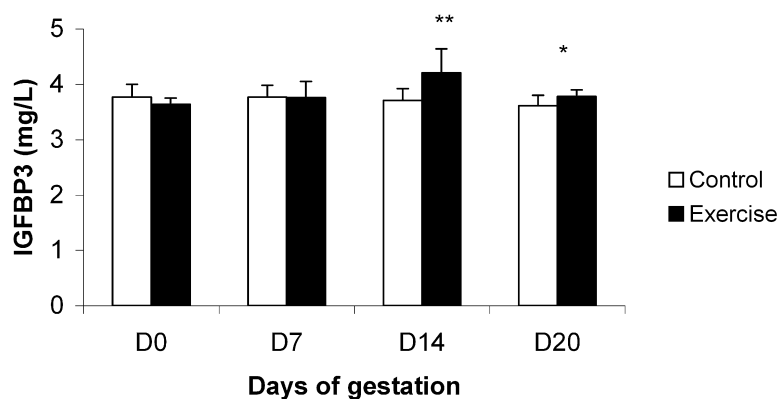


Fig. 3. Changes in the plasma levels of insulin-like growth factor binding protein-3 (IGFBP-3) during pregnancy in controls and exercise group.
* $p < 0.05$, ** $p < 0.01$.

D14 were 16.53 ± 2.2 pg/ml and 23.25 ± 3.28 pg/ml, respectively. The difference in this parameter between the two groups was significant ($p < 0.01$, Fig. 1). Plasma IGF-I levels of controls and exercise group on the D0, D7 and D14 did not show any significant difference. Only the IGF-I levels of exercise groups on the D20 (27.99 ± 3.8 ng/ml) was significant compared to control (24.42 ± 1.64 ng/ml) ($p < 0.05$, Fig. 2). Plasma IGFBP-3 levels of exercise group on the D14 and D20 were 4.21 ± 0.42 and 3.78 ± 0.12 mg/liter while those of controls were 3.71 ± 0.21 and 3.61 ± 0.19 mg/liter, respectively. The difference in this parameter on these days between the two groups was

significant ($p < 0.01$ for D14, $p < 0.05$ for D20, Fig. 3).

DISCUSSION

Recent studies have shown that moderate physical exercise practiced before and during pregnancy, did not affect fetal development in rats (Houghton et al. 2000) and in humans (Clapp et al. 2002) while strenuous antenatal exercise caused reduced birth weight, umbilical cord length and placental weight (Ribeiro et al. 1991). These conflicting results about the effects of maternal exercise on the fetus are probably due to the different exercise intensities to which the

mothers were submitted, to animal familiarity with the physical exercise or to different species of animals used (Clapp et al. 2000). We think that the exercise performed on the study group may be responsible for the decrease in the fetal birth weights and fetal liver weights. Because of the relatively high blood circulation of rat muscles functioning during exercise, fetuses might have relatively low blood circulation and in turn weight of fetus at birth and fetal liver might decrease. The training program consisted of running at 20 m/min for 20 min/day per day for 19 days in exercise group in this study. In a study performed on pregnant Sprague-Dawley rats (Houghton et al. 2000), the exercise protocol consisted of treadmill running at 30 m/min, for 60 min, 5 days per week, for 4 weeks prior to conception, continued until the day 19 of pregnancy. In their study, exercise caused a significant reduction in fetal body weight, placental weight, and fetal organ weights (heart, kidney, brain, and liver) compared to sedentary control animals. In contrast, found decreases only in the weights of fetuses and fetal liver even though the exercise we applied was milder than that used in the previous study (Houghton et al. 2000). The milder exercise might be the reason for the normal weights of fetal heart and placenta in our study. Likewise, numbers and heights of fetuses, fetal kidney weights and lengths of umbilical cord did not change in our study with exercise. A moderate exercise in mid and late pregnancy symmetrically reduces fetoplacental growth whereas a reduction in exercise volume enhances fetoplacental growth with a proportionally greater increase in fat mass than in lean body mass (Clapp et al. 2002). The interactions amongst maternal exercise, fetal oxygenation and fetoplacental growth are complex because the effects of exercise on the maternal physiological parameters of interest (placental bed blood flow and arterial blood sugar and oxygen content) vary with the type, frequency, duration and intensity of the exercise as well as the physical fitness of the mother and the time point in the pregnancy when the exercise is carried out (Clapp et al. 2000, 2002). Normal placental development is linked to its functional capacity

that is an important determinant of fetal growth potential. Indeed, many of its morphometric characteristics correlate directly with size at birth (Jackson et al. 1995).

IGF-I can act in an endocrine as well as an autocrine/paracrine fashion and has both metabolic (insulin-like) and anabolic (growth) functions (Jones and Clemmons 1995). IGF-I and the IGFBP-3 play a role in the regulation of late fetal growth and development (Klauwer et al. 1997). Plasma GH, IGF-I and IGFBP-3 levels of exercise group were increased, especially at late gestational period (on the D14 and D20) in our study compared to controls. IGF-I is known to be an important growth factor during intrauterine life (Verhaeghe et al. 1993). For this reason plasma IGF-I was analyzed together with IGFBP-3, which was the major carrier of IGF-I (approximately 80%), during all period of pregnancy with exercise. The plasma GH levels of exercise group increased significantly on the D14 (Fig. 1). The increase in plasma GH after exercise has been well documented (Galbo 1985; Flanagan et al. 1997; Wallace et al. 1999). Plasma GH levels of controls showed no changes during pregnancy compared to on the D0 while those of exercise group were significantly higher on D14 ($p < 0.05$).

Plasma IGF-I response to physical exercise has not been demonstrated consistently; some research groups have observed increments, while others showed no variations (Di Luigi et al. 1997; Nguyen et al. 1998; Wallace et al. 1999; Turgut et al. 2003). This may be due to the different type and duration of the performance and training conducted. In fact, intense endurance training increased IGF-I (Roelen et al. 1997), but the intensity, duration and frequency of the exercise training could be determined in GH/IGF-I axis responses. Similarly, two investigations have shown (Poehlman et al. 1994; Roelen et al. 1997) that endurance-training results in elevated resting levels of serum total IGF-I in healthy young and old individuals. We investigated plasma IGF-I levels of pregnant rats on the D0, D7, D14 and D20, and found no significant change in the IGF-I levels of both control and exercise groups on the following days compared to those of the D0 in

each group. When we compared plasma IGF-I levels between groups there were no significant changes on the D0, D7 and D14 while the levels of exercise group on the D20 increased significantly compared to controls (Fig. 2).

It appears that an increase in the plasma levels of IGF-I occurs especially in the late period of pregnancy with exercise. Several authors have described an increase in plasma IGF-I after endurance training (Poehlman et al. 1994; Koziris et al. 1999), but others have reported an opposite effect (Eliakim et al. 1996, 1998).

IGF-I has been thought to regulate levels of IGFBP-3 (Binoux 1997). Moreover, exercise and training have been reported to increase IGFBP-3 levels (Nguyen et al. 1998; Koziris et al. 1999), but not in other study (Deuschle et al. 1998). In our study, plasma IGFBP-3 levels of pregnant rats having exercise were significantly higher on D14 and D20 than those of controls ($p < 0.01$, Fig. 3); plasma IGFBP-3 levels increase in second half of the pregnancy. However, investigations performed to evaluate possible changes in IGFBP-3 after physical exercises have provided equivocal results (Cohich and Clemmons 1993; Schwarz et al. 1996).

A significant increase in plasma GH, IGF-I and IGFBP-3 levels was observed in pregnant rats with exercise, which did not lead to any increase in the weights of fetus in exercise group. In contrast, there was a decrease in the weights of the fetuses of exercise group. This might be due to the overflow of blood to extremities rather than fetuses during exercises. Thus, the positive effects of GH, IGF-I and IGFBP-3 on fetal growth might have been masked owing to the reduced blood circulation of placenta. In order to clarify this issue, further investigations are needed.

CONCLUSION

We have shown that exercise increases the plasma levels of GH, IGF-I and IGFBP-3 in pregnant rats towards the end of pregnancy. Conversely, maternal exercise decreased the weights of fetus and fetal liver, but did not affect the number and heights of fetuses, fetal heart and kidney weights, umbilical cord lengths and pla-

centa weights. The level of exercise employed in this study caused adverse effects on fetal growth. Thus, severe exercise is not recommended during pregnancy.

References

- Ballard, D.C., Baxter, R., Binoux, M., Clemmons, D., Drop, S., Hall, K., Hintz, R., Rechler, M., Rutanen, E. & Schwander, J. (1989) On the nomenclature of the IGF binding proteins. *Acta Endocrinologica*, **121**, 751-752.
- Bell, R. & Palma, S. (2000) Antenatal exercise and birthweight. *Aust. N. Z. J. Obstet. Gynaecol.*, **40**, 70-73.
- Binoux, M. (1997) GH, IGFs, IGF-binding protein-3 and acid-labile subunit: what is the pecking order? *Eur. J. Endocrinol.*, **137**, 605-609.
- Clapp, J.F., Stepanchak, W., Kortan, M. & Fanselow, S. (2000) Portal vein blood flow-effects of pregnancy, gravity, and exercise. *Am. J. Obstet. Gynecol.*, **183**, 167-172.
- Clapp, J.F., Kim, H., Burciu, B., Schmidt, S., Petry, K. & Lopez, B. (2002) Continuing regular exercise during pregnancy: Effect of exercise volume on fetoplacental growth. *Am. J. Obstet. Gynecol.*, **186**, 142-147.
- Cohich, W.S. & Clemmons, D.R. (1993) The insulin-like growth factors. *Annu. Rev. Physiol.*, **55**, 131-153.
- Collett-Solberg, P.F. & Cohen, P. (1996) The role of insulin-like growth factor binding proteins and the IGFBP proteases in modulating IGF action. *Growth Disord.*, **25**, 591-609.
- Deuschle, M., Blum, W.F., Frystyk, J., Orskov, H., Schweiger, U., Weber, B., Korner, A., Gotthardt, U., Schmider, J., Standhardt, H. & Heuser, I. (1998) Endurance training and its effect upon the activity of GH-IGFs system in elderly. *Int. J. Sports Med.*, **19**, 250-254.
- Di Luigi, L., Conti, F.G., Casini, A., Guidetti, L., Zeppe, G., Pigozzi, F., Spera, G., Fortunio, G. & Romanelli, F. (1997) Growth hormone and insulin-like growth factor I responses to moderate submaximal acute physical exercise in man: effects of octreotide, a somatostatin analogue, administration. *Int. J. Sports Med.*, **18**, 257-263.
- Eliakim, A., Brasel, J.A., Mohan, S., Barstow, T.J., Berman, N. & Cooper, D.M. (1996) Physical fitness, endurance training, and the growth hormone-insulin-like growth factor I system in adolescent females. *J. Clin. Endocrinol. Metab.*, **81**, 3986-3992.
- Eliakim, A., Brasel, J.A., Mohan, S., Wong, W.L.T. & Cooper, D.M. (1998) Increased physical activity and the growth hormone-IGF-I axis in adolescent males. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **275**, R308-R314.
- Flanagan, D.E., Taylor, M.C., Parfitt, V., Mardell, R., Wood, P.J. & Leatherdale, B.A. (1997) Urinary growth hormone following exercise to assess growth hormone production in adults. *Clin. Endocrinol. (Oxf.)*, **46**, 425-429.
- Galbo, H. (1985) The hormonal response to exercise. *Proc. Nutr. Soc.*, **44**, 257-266.
- Houghton, P.E., Mottola, M.F., Plust, J.H. & Schachter, C.L. (2000) Effect of maternal exercise on fetal and placental glycogen storage in the mature rat. *Can. J. Appl. Physiol.*, **25**, 443-452.
- Isaksson, O.G., Ohlsson, C., Nilsson, A., Isgaard, J. & Lindahl, A. (1991) Regulation of cartilage growth by growth hormone and insulin-like growth factor I. *Pediatr. Nephrol.*, **5**, 451-453.
- Jackson, M.R., Gott, P., Lye, S.L., Ritche, J.W.K. & Clapp, J.F.

- (1995) The effects of maternal aerobic exercise on human placental development: placental volumetric composition and surface areas. *Placenta*, **16**, 179-191.
- Jenkins, P.J. (1999) Growth hormone and exercise. *Clin. Endocrinol. (Oxf.)*, **50**, 683-689.
- Jones, J.I. & Clemmons, D.R. (1995) Insulin-like growth factors and their binding proteins: biological actions. *Endocr. Rev.*, **16**, 3-34.
- Klauwer, D., Blum, W.F., Hanitsch, S., Rascher, W., Lee, P.D. & Kiess, W. (1997) IGF-I, IGF-II, free IGF-I and IGFBP-1, -2 and -3 levels in venous cord blood: relationship to birth-weight, length and gestational age in healthy newborns. *Acta Paediatr.*, **86**, 826-833.
- Koistinen, H., Koistinen, R., Selenius, L., Ylikorkkala, O. & Seppala, M. (1996) Effect of marathon run on serum IGF-I and IGF-binding protein 1 and 3 levels. *J. Appl. Physiol.*, **80**, 760-764.
- Koziris, L.P., Hickson, R.C., Chatterton, R.T., Groseth, R.T., Christie, J.M., Goldflies, D.G. & Unterman, T.G. (1999) Serum levels of total and free IGF-I and IGFBP-3 are increased and maintained in long-term training. *J. Appl. Physiol.*, **86**, 1436-1442.
- Nguyen, U.N., Mougín, F., Simon Rigaud, M.L., Rouillon, J.D., Marguet, P. & Regnard, J. (1998) Influence of exercise duration on serum insulin-like growth factor and its binding proteins in athletes. *Eur. J. Appl. Physiol.*, **78**, 533-537.
- Ogilvy-Stuart, A.L., Hands, S.J., Adcock, C.J., Holly, J.M., Matthews, D.R., Mohamed-Ali, V., Yudkin, J.S., Wilkinson, A.R. & Dunger, D.B. (1998) Insulin, insulin-like growth factor I (IGF-I), IGF-binding protein-1, growth hormone, and feeding in the newborn. *J. Clin. Endocrinol. Metab.*, **83**, 3550-3557.
- Poehlman, E.T., Rosen, C.J. & Copeland, K.C. (1994) The influence of endurance training on insulin-like growth factor-I in older individuals. *Metabolism*, **43**, 1401-1405.
- Rajaram, S., Baylink, D.J. & Mohan, S. (1997) Insulin-like growth factor-binding protein in serum and other biological fluids: regulation and functions. *Endocr. Rev.*, **18**, 801-831.
- Ribeiro, C.A.L., Maia Campos, G., Melis, M.S., Sala, M.A. & Lopes, R.A. (1991) Alteraciones morfológicas y estereológicas del epitelio lingual de fetos de rata provocadas por el ejercicio materno. *Archivos de la Facultad de Medicina de Zaragoza*, **30**, 84-90. (in Turkish)
- Riemann, M.K. & Kanstrup Hansen, I.L. (2000) Effects on the fetus of exercise in pregnancy. *Scand J. Med. Sci. Sports*, **10**, 12-19.
- Roelen, C.A., De Vries, W.R., Koppeschaar, H.P., Vervoorn, C., Thijssen, J.H. & Blankenstein, M.A. (1997) Plasma insulin-like growth factor-I and high affinity growth hormone-binding protein levels increase after 2 weeks of strenuous physical training. *Int. J. Sports Med.*, **18**, 238-241.
- Rosen, C.J. & Pollak, M. (1999) Circulating IGF-I: new perspectives for a new century. *Trends Endocrinol. Metab.*, **10**, 136-141.
- Schwarz, A.J., Brasel, J.A., Hintz, R.L., Mohan, S. & Cooper, D.M. (1996) Acute effect of brief, low and high-intensity exercise on circulating insulin-like growth factor (IGF), I, II, and IGF-binding protein-3 and its proteolysis in young healthy men. *J. Clin. Endocrinol. Metab.*, **81**, 3492-3497.
- Turgut, G., Kaptanoğlu, B., Turgut, S., Genç, O. & Tekintürk, S. (2003) Influence of acute exercise on urinary protein, creatinine, insulin-like growth factor-I (IGF-I) and IGF Binding Protein-3 concentration in children. *Tohoku J. Exp. Med.*, **201**, 165-170.
- Verhaeghe, J., van Bree, R., van Herck, E., Laureys, J., Bouillon, R. & van Assche, F.A. (1993) C-peptide, insulin-like growth factors I and II, and insulin-like growth factor binding protein-1 in umbilical cord serum: Correlations with birth weight. *Am. J. Obstet. Gynecol.*, **169**, 89-97.
- Wallace, J.D., Cuneo, R.C., Baxter, R., Orskov, H., Keay, N., Pentecost, C., Dall, R., Rosen, T., Jorgensen, J.O., Cittadini, A., Longobardi, S., Sacca, L., Christiansen, J.S., Bengtsson, B.A. & Sonksen, P.H. (1999) Responses of the growth hormone (GH) and insulin-like growth factor axis to exercise, GH administration, and GH withdrawal in trained adult males: a potential test for GH abuse in sport. *J. Clin. Endocrinol. Metab.*, **84**, 3591-3601.
- Well, S. (1998) Growth hormone and insulin-like growth factor I as anabolic agents. *Curr. Opin. Clin. Nutr. Metab. Care*, **1**, 257-262.