The Preferential Expression pattern of insulin-like growth factor -1 and its isoforms in the gastrocnemius muscle suggests preferential roles in the running and Sedentary Adolescents

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Introduction

Insulin-like growth factor-1 (IGF-I) is a potent mitogen which promotes cellular proliferation and prevents apoptosis in normal and cancer cells (Yu and Ro- han, 200). Thus it is involved in the growth, development and regulation of homeostasis in a tissue-specific manner (Stewart and Rotwein, 1996).

Human (Castaneda et al., 2000; Marcell et al., 2001) and animal (Edwall et al., 1989; Ullman et al., 1990) studies suggest that IGF-1 is an important regu- lator of skeletal muscle growth. Circulat- ing IGF-1 is required for muscle and bone growth in adolescence and for the maintenance of muscle mass and strength in the adolescent period as well as in adulthood. Serum level of IGF-1 not only affects proliferation of peripheral tissues but also affects CNS development and proliferation (Jeon and Ha, 2014). For example serum IGF-1 induces neuro- genesis during brain development and has an important role in repair processes of traumatic brain (Madathil and Saat- man, 2014). It was found that this IFG-1- induced neurogenesis is enhanced by exercise (Trejo et al., 2002). IGF-1 in the blood reach its peak during adolescence and is maintained at somewhat lower levels during adulthood (Hameed et. al., 2002).

Alternative splicing, multiple tran- scription initiation sites and different polyadenylation signals give rise to di- verse mRNA isoforms for IGF-1 in different tissues, such as IGF-1Ea (Jansen et al., 1983; Barton, 2006), IGF-1Eb (Rotwein, 1986) and IGF-1Ec (Philippou et al., 2009) transcripts.

In the skeletal muscles, in addition to IGF-1, at least two isoforms of the IGF

-I gene have recently been shown to be expressed by animal muscles when sub-

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jected to mechanical stimulation (Yang et al., 1996; McKoy et al., 1999).

The first isoform of IGF-I is called IGF-IEa and is cloned and detected to be expressed by the skeletal muscle (Yang et al., 1996; Mckoy et al., 1999; Hameed et al., 2003). Increasing expression of IGF-Ea in the mouse has been shown to produce an anabolic effect on the skeletal muscle (Barton-Davies et al., 1998). In transgenic mouse model, IGF-1Ea over- expression of this isoform in the skeletal muscle, was shown to have pronounced muscle hypertrophy (Musaro et al., 2001).

The second isoform, IGF-1Ec, also called mechano-growth factor (MGF), is a splice variant of IGF-1, which has been shown *in vitro* as well as *in vivo* to in- duce growth and hypertrophy in mechanically stimulated or damaged muscle in animals (Yang et al., 1996). IGF-1 isoforms display activities not shown by the parent IGF-1 (Matheny et al., 2010).

Because of the importance of IGF-1 and its isoforms in health and disease, there is increasing interest in the studies on the expression pattern of this hormone and its isoforms in humans. In this study the expression of the IGF-1 and IGF-1 isoforms in sedentary and running adolescents were investigated in details in the serum and the gastrocnemius muscle .

Subjects And Methods

Ethical approval:

A written informed consent was obtained by all the volunteers to partici- pate in this study, which was approved by the Ethics Committee of the National and international laws. All experimental procedures conformed to the Declaration of Helsinki.

Subjects:

Group I: Twenty healthy and sedentary males participated in the study (Students of Faculty of Engineering; Minia University). They had not participated in any type of training or any regular exercise regime for at least 6 months before the study.

Group II: Twenty healthy adolescent males participated in this study (Students of Faculty of Physical Education; Minia University). They are running for 5 days/ week for at least 5 years for 1200 meters each time.

For both groups, the age was 17±1.5 years, height was 170 ± 5.5 cm, body mass was 70.8±2.4 kg, and body mass index was 23.6±0.5. These individ- uals refrained from taking any medica- tions or nutritional supplementations a week before taking the blood and muscle samples. They were also instructed to maintain their habitual diet. On the day prior to and the day of biopsy and blood draw they were asked to have similar meals.

Blood sampling and serum measure- ments.

Blood samples were withdrawn just after running set for the running group and at the same time for sedentary group. Blood was collected in vacutainer tubes, centrifuged and serum were stored frozen (-80°C). Serum IGF-1, IGF-1Ea and IGF
-1Ec (MGF) were determined by a stand- ard sandwich enzyme-linked immuno-sorbent assay (ELISA) protocol using a commercially available kit (Assay De- signs, Michigan, USA) according to the manufacturer's instructions. All samples were run simultaneously, analyzed in triplicate and the results were averaged. According to the manufacturers, the min- imal detection limits of the assay used for were 34.2 pg/ml.

Muscle biopsies and tissue processing.

According to Philippou et. al. (2008) the skeletal muscle biopsies were obtained from the middle portion of the gastrocnemius muscle under local anes- thesia (2% lidocaine; AstraZeneca, Lon-don, UK) from both groups. A 5-mm Bergstrom biopsy needle was inserted at a constant depth using the percutaneous needle biopsy technique. The muscle sample (~70-100 mg) obtained from each biopsy was divided into two pieces. The first piece was snap-frozen in liquid ni-trogen and then stored at -80°C until ana- lyzed for RNA and protein content. The second piece was fixed in formaldehyde (10% final concentration) for subsequent immuno-histochemical analysis.

RNA extraction and semi-quantitative RT-PCR analysis.

After Philippou et al., (2008), each muscle sample was homogenized and total RNA was extracted using Trizol Reagent (Invitrogen

Corp., Carlsbad, CA, USA) according to the manufacturer's recommendations. The extracted RNA was dissolved in diethy lpyrocarbonate (DEPC)- treated water and the concentra- tion and purity were determined spectro- photometrically (Genova, Jenway, Essex, UK) by absorption at 260 and 280 nm. The quality and integrity of total RNA were assessed by visual inspection of the electrophoretic pattern of 18S and 28S ribosomal RNA in ethidium bromide- stained 1% agarose gels under ultraviolet (UV) light and electrophoresis of the RNA confirmed that it was intact. The RNA samples were used for the determi- nation of the mRNA of specific IGF-1 isoforms by reverse transcription and relative quantitative RT-PCR procedures. Both the RT and PCR methods used in the present study have been described and extensively validated in previous publications concerning exercise mRNA responses (Bickel et al., 2005; Kim et al., 2005). Primer sets for IGF-1, IGF- 1Ea and IGF-1Ec (MGF) were previous- ly used by Yang et al., (1996); Bickel et al., (2005) and Hameed et al., (2003), respectively. Each set of primers was designed to lie within different exons of the IGF-1 gene and to detect and amplify specifically either IGF-1 mRNA only or one of its transcripts (namely IGF-1Ea, and IGF-1Ec or MGF). All target se- quences were identified by sequencing analysis to ensure specificity of the pri- mers and to further verify each tar- get IGF-1 mRNA.

Protein extraction and Western analy- sis.

Total proteins were extracted from the same muscle biopsy sample used for total RNA isolation using the Trizol Re- agent protocol. The extracts were ana- lyzed for total protein concentration us- ing the Bradford procedure (Bio-Rad Protein Assay, Hercules, CA, USA). Samples were stored in aliquots at -80°C until Western blot analysis as previously described (Philippou et al. 2008). The following primary antibodies were used for the immunodetection of MGF using a rabbit anti-human IGF-1Ec (MGF) poly- clonal antibody (1:10,000 dilution), which was raised against a synthetic peptide corresponding to the last 24 ami- no acids of the E domain of human MGF, as has been

described elsewhere (Philippou et al. 2008). Mouse IGF-1Ea, monoclonal and anti-IGF-1 (Clone-M23) (1:1,000 dilution) (MS-1508, Ne- oMarkers, Fremont, CA, USA; molecu- lar weight of antigen: ~21 kDa) were used. It should be remarked that the bands detected in Western blot analyses by these antibodies represent the (full length) IGF-1, IGF-1Ea, and IGF-1Ec (Philippou et al., 2008). After the over- night incubation of blots with the prima- ry antibodies, membranes were incubat- ed with a horseradish peroxidase- conjugated secondary anti-rabbit IgG (goat anti-rabbit, 1:2,000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA) or anti-mouse IgG goat anti- mouse, 1:2,000 dilution; Santa Cruz Bio- technology), for 1 h at room tempera- ture. Glyceraldehyde 3-phosphate dehy- drogenase (GAPDH) was used as an internal control to correct for potential variation in the protein loading and to normalize the protein measurements on the same immunoblot. Blots were incu- bated with a mouse monoclonal primary antibody for GAPDH (1:2,000 dilution; Santa Cruz Biotechnology) and with a horseradish peroxidase-conjugated sec- ondary anti-mouse IgG (goat anti-mouse, 1:2,000 dilution; Santa Cruz Biotechnol- ogy). Specific band (s) were visualized by exposure of the membrane to x-ray film, after incubation with an enhanced chemiluminecent (ECL) substrate ac- cording to the manufacturer's protocol (SuperSignal; Pierce Biotechnology, Rockford, IL, USA). The films were captured under white light in a Kodak EDAS 290 imaging system (Carestream Health, Inc. Rochester, NY, USA) and proteins were quantified by band densi- tometry using image software (Scientific Imaging Systems, Kodak ID, New Ha- ven, CT, USA) (Philippou et. al., 2008).

Statistical analysis

Data were given as means± SEM. Comparisons were made using a paired *t* -test. The level of statistical significance was accepted at $P<0.05$

Immuno-histochemical analysis.

Formaldehyde-fixed skeletal mus- cle samples were paraffin wax embedded and processed for paraffin sections. Mi- crotome sections of 3 μm were allowed to adhere to glass slides, dried at 37°C overnight,

dewaxed in xylene and rehydrated in serial dilutions of ethanol. The sections were then incubated with the same primary antibodies used for the Western blot analyses, *i.e.* the polyclonal anti-IGF-1Ea and anti-IGF-1Ec (MGF) antibodies at a dilution of 1:1,000 in PBS and the monoclonal anti-IGF-1 (1:50 dilution, MS-1508; NeoMarkers) overnight at 4°C. Secondary biotinylated goat anti-rabbit IgG or goat anti-mouse IgG (Dako Real EnVision, Glostrup, Denmark) was then added and tissue sections were visualized under light mi- croscopy. Negative control staining pro- cedures were included in all immuno-histochemical analyses, as described elsewhere (Philippou et al., 2008).

results

serum igf-1, igf-1ea and igf-1ec by eliza technique

in Table 1: mean ±SEM of serum IGF-1 in the running group is 450±19.4 ng/ml and this level is significantly higher than that of sedentary group (mean= 270± 17.2 SEM) (P value <0.03). Mean

serum IGF-1Ea are 120 ng/ml in the sedentary group and 132 ng/ml in the running group and these levels showed non-significant differences between the studied groups (histogram in the lower panel). The mean of IGF-1Ec in the run- ning group is much higher (378±15.2 ng/ ml) than that of sedentary group (98±12.4 ng/ml) (p value <0.002) (histogram in the lower panel). Semi-quantitative RT-PCR in gas- trocnemius muscle biopsy (Fig. 1A), shows semi-quantitative RT-PCR gel images which demonstrate the expression of the *IGF-1* transcripts and its isoforms *IGF-1Ea* and *IGF-1Ec* mRNA for 5 of the studied individual volunteers with sedentary life style. In (Fig. 1B) RT- PCR gel images that show the expression of the IGF-1 transcripts and IGF-1Ea and IGF-1Ec transcripts of 5 of the studied individual volunteers of running group. In (Fig. 1C), densitometric analysis is showing the relative mRNA levels (corrected for 18S) in the two groups. The histogram shows the values (mean±SEM.; n=20 for each group) were normalized to each corresponding ribosomal 18S and ex- pressed as percentage changes (%) of mRNA levels in both groups. IGF-1 is significantly higher in the running group than the resting group (P value < 0.03). *IGF-1Ea* is moderately expressed in both groups but there are no significant differ- ences

between the two groups (P value 0.19). *IGF-1Ec*, is significantly much higher in the running group than that of sedentary group (P value <0.002).

Western blot analysis of the gas- trocnemius muscle biopsy

Immunoblot images of IGF-1, IGF- 1Ea and IGF-1Ec proteins in the seden- tary group (Fig. 2A) and the running group (Fig. 2B). (Fig. 2C), shows im- munoblotting quantification. The histo- gram shows the values (means ±SEM.; n=20 for each group) which are normal- ized to each corresponding GAPDH on the same immunoblot and expressed as percentage changes (%). In (Fig. 2C) IGF-1 and IGF-1Ec proteins are signifi- cantly higher in the running group than the sedentary group (P values <0.02 and <0.01 respectively). Note that IGF-1Ea protein is lower in the running group than the sedentary group (P value < 0.04).

Immunohistochemistry of IGF-1 and its isoforms in gastrocnemius muscle

Sedentary group figures shows samples of the Gastrocnemius muscle stained with anti-IGF-1 antibody in (Fig. 3A) and with anti-IGF-1Ea in (Fig. 3B) and anti- IGF-1Ec (Fig. 3C). Moderate brown color staining are shown for both IGF-1 and IGF-1Ea in the sedentary group (Fig. 3A, 3B). While using anti- IGF-1Ec (MGF) antibody in (Fig. 3C) the samples appear mildly stained with anti-IGF-1Ec. Note also the presence of negatively stained muscle cells for IGF- 1Ec among the week or mild positive cells in this group (Fig. 3C). Specificity of the immuno-histochemical detections was confirmed by the absence of immu-noreactivity in the negative control sec- tions in (Fig. 3D. 3E, 3F)

In the running group: figures of Gastrocnemius muscle of an individual that are shown stained with anti-IGF-1 in (Fig. 4A) with anti-IGF-1Ea in (Fig. 4B) and with anti-IGF-1Ec (MGF) in (Fig. 4C) antibodies. There is moderate stain- ing for IGF-1 (Fig. 4A) but higher than the sedentary group. For IGF-1Ea there is mild staining in this group (Fig, 4B). Note that the sample appear densely stained for IGF-1Ec (much more than the previous group for IGF-1Ec)in (Fig.4C). Specificity of the immuno-histochemical detections was confirmed by the absence of immunoreactivity in the negative control sections in (Fig. 4D. 4E, 4F)

Table (1)

Serum IGF-1 and its isoforms in the study groups by ELISA technique in ng/ ml

	IGF-1	IFG-1Ea	IGF-1Ec
Sedentary group Mean ± SEM	270 ± 17.2	120 ± 30.1	98 ± 12.4
Running group Mean ± SE	450 ± 19.4	132 ± 15	378 ± 15.2
P value	**< 0.03 S**	**0.19 NS**	**<0.002 S**

S= significant NS = non significant

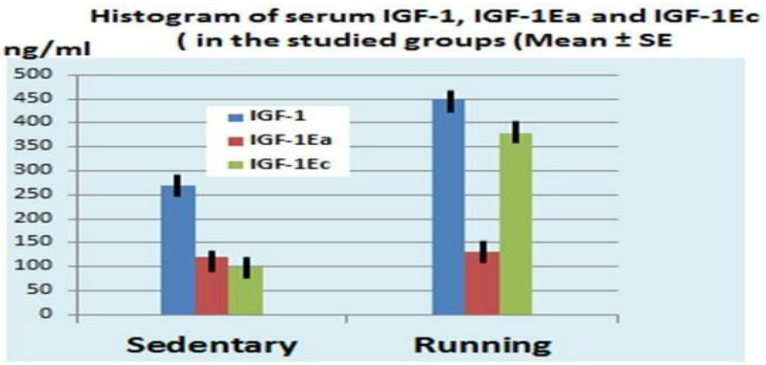


Fig.1. Transcriptional changes of IGF-1& its isoforms mRNA in the gastrocnemius muscle biopsy in the studied groups: (A), shows semi- quantitative RT-PCR gel images which demonstrate the expression of the IGF-1 and its isoforms IGF-1Ea and IGF-1Ec mRNA for 5 of the studied individual volunteers with sedentary life style. In (B), RT- PCR gel images that show the expression of the IGF-1 and IGF-1Ea and IGF-1Ec transcripts of 5 of the stud- ied individual volunteers of running group. In (C), Densitometric analysis showing the relative mRNA levels (corrected for 18S) in the two groups. The histogram shows the values (mean±SEM.; n=20 for each group) were normalized to each corresponding ribosomal 18S and expressed as percent- age changes (%) of mRNA levels in both groups. IGF-1 is significantly higher in the running group than the resting group (P value < 0.03). IGF-1Ea is moderately expressed in both groups but there are no significant differences between the two groups (P value 0.19). For IGF-1Ec, it is significantly

higher in the running group than that of sedentary group (P value <0.002).

Fig. 2 Western blot analysis of IGF1, IGF-1Ea and IGF-1Ec (MGF) in the gastrocnemius muscle biopsy in the studied groups: Immunoblot images of IGF-1, IGF-1Ea and IGF-1Ec proteins in the sedentary group (A) and the run- ning group (B). (C), shows immunoblot- ting quantification. The histogram shows the values (means±SEM; n=20 for each group) which are normalized to each corresponding GAPDH on the same im- munoblot and expressed as percentage changes (%). IGF-1 and IGF-1Ec pro- teins are significantly higher in the run- ning group than the sedentary group (P values <0.02 and <0.01 respectively). Note that IGF-1Ea is lower in the run- ning group than in the sedentary group (P value < 0.04).

Fig. 3 Immunohistochemistry of IGF-1 and its isoforms in gastrocnemi- us muscle of the sedentary group: Fig- ures represent gastrocnemius muscle samples of an individual from sedentary group that are stained with anti-IGF-1 in (A) anti-IGF-1Ea in (B) and anti-IGF- 1Ec in (MGF) (C) antibodies. Note that the samples appear moderately stained for both IGF-1 and IGF-1Ea, while the samples appear mildly stained with anti- IGF-1Ec. Note also the presence of neg- atively stained muscle cells for IGF-1Ec among the week or mild positive cells in this group (C). Specificity of the immu- no-histochemical detections was con- firmed by the absence of immunoreactiv- ity in the negative control sections in (D, E and F) (scale bar 200 um).

Fig. 4 Immunohistochemistry of IGF-1 and its isoforms in gastrocnemi- us muscle of the running group: Fig- ures represent gastrocnemius muscle samples of an individual from running group that are stained with anti-IGF-1 in (A) anti-IGF-1Ea in (B) and anti-IGF- 1Ec in (MGF) (C) antibodies. There is moderate staining for IGF-1. For IGF-1Ea there is mild staining in this group which is less than that of the resting group. Note that the sample appear high- ly stained for IGF-1Ec (much more than the previous group for IGF-1Ec). Speci- ficity of the immuno-histochemical de- tections was confirmed by the absence of immunoreactivity in the negative control sections in (D. E, F) (scale bar 200 um).

Fig. (1)

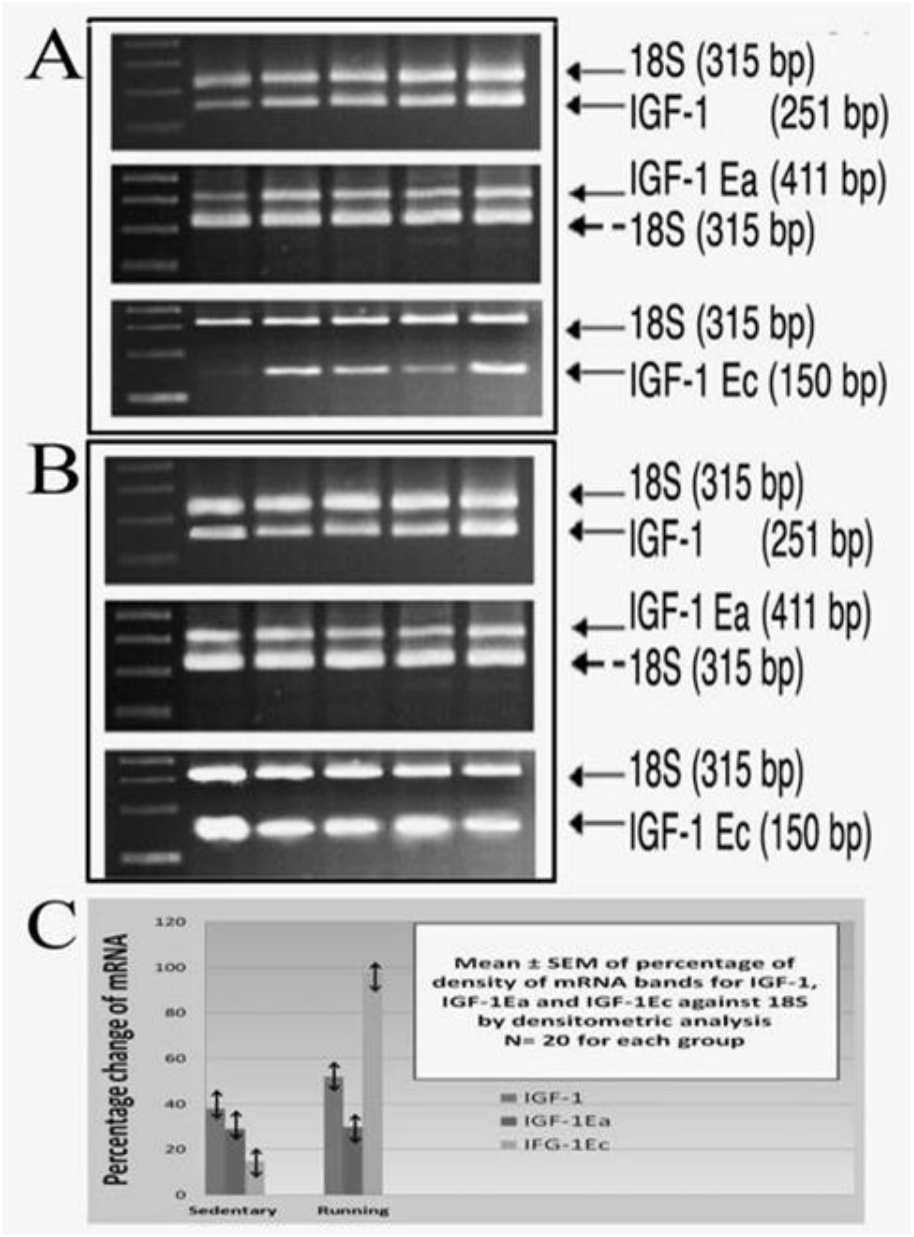

A
18S (315 bp)
IGF-1 (251 bp)
IGF-1 Ea (411 bp)
18S (315 bp)
18S (315 bp)
IGF-1 Ec (150 bp)
B
18S (315 bp)
IGF-1 (251 bp)
IGF-1 Ea (411 bp)
18S (315 bp)
18S (315 bp)
IGF-1 Ec (150 bp)
C
Percentage change of mRNA
120
100
80
60
40
20
0
Sedentary
Running
Mean ± SEM of percentage of density of mRNA bands for IGF-1, IGF-1Ea and IGF-1Ec against 18S by densitometric analysis N= 20 for each group
IGF-1
IGF-1Ea
IFG-1Ec

Fig. (2)

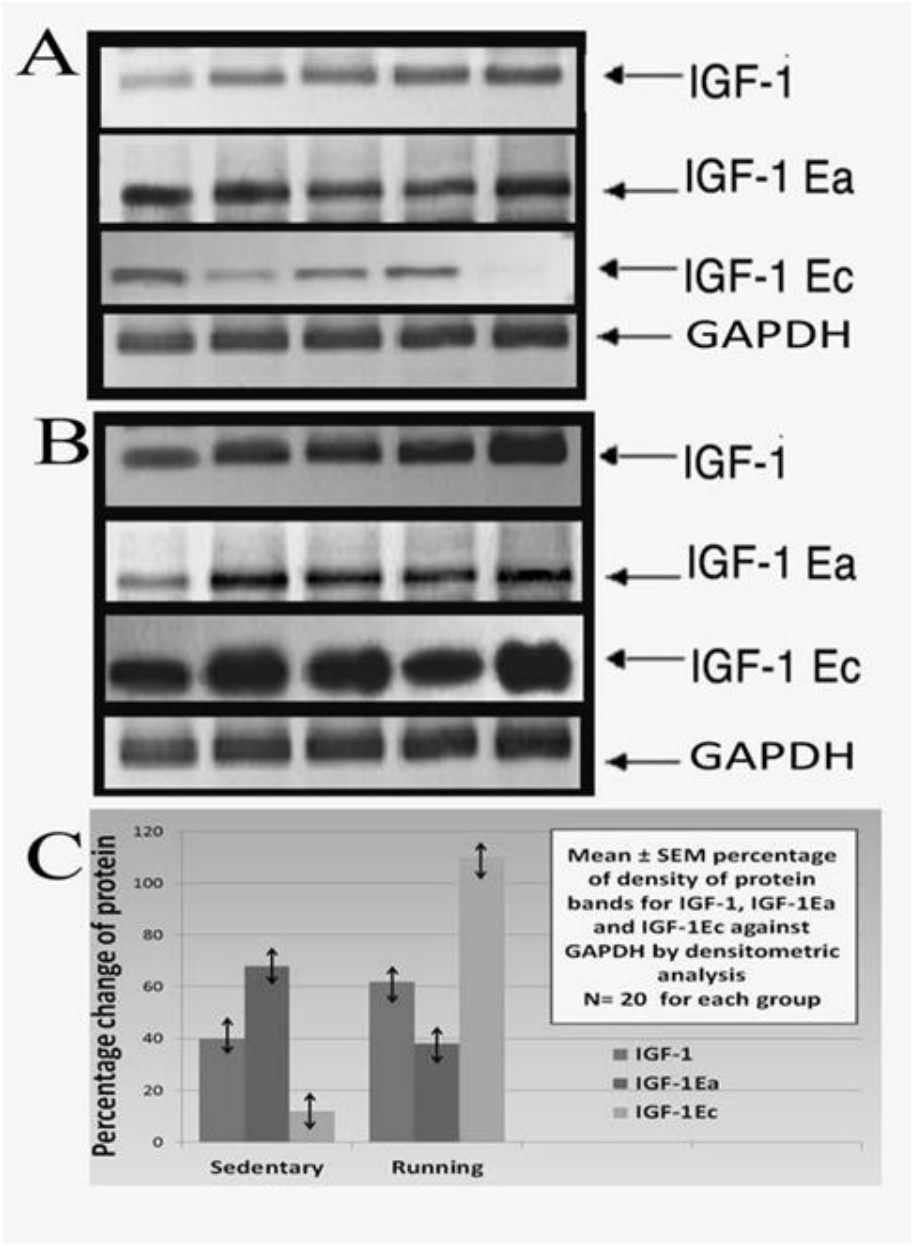

A
IGF-1
IGF-1 Ea
IGF-1 Ec
GAPDH
B
IGF-1
IGF-1 Ea
IGF-1 Ec
GAPDH
C
Percentage change of protein
120
100
80
60
40
20
0
Sedentary
Running
Mean ± SEM percentage of density of protein bands for IGF-1, IGF-1Ea and IGF-1Ec against GAPDH by densitometric analysis
N= 20 for each group
IGF-1
IGF-1Ea
IGF-1Ec

Fig. (3)

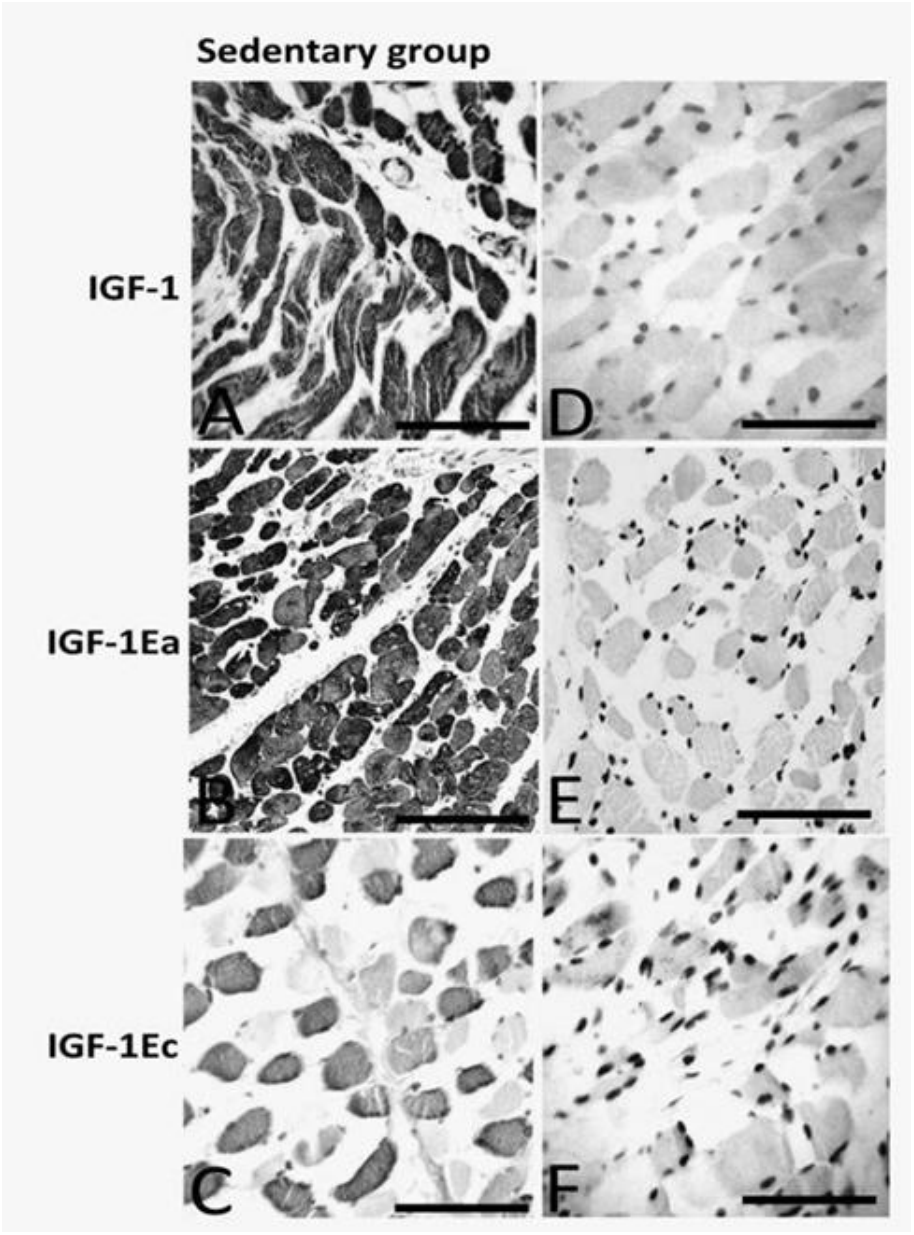
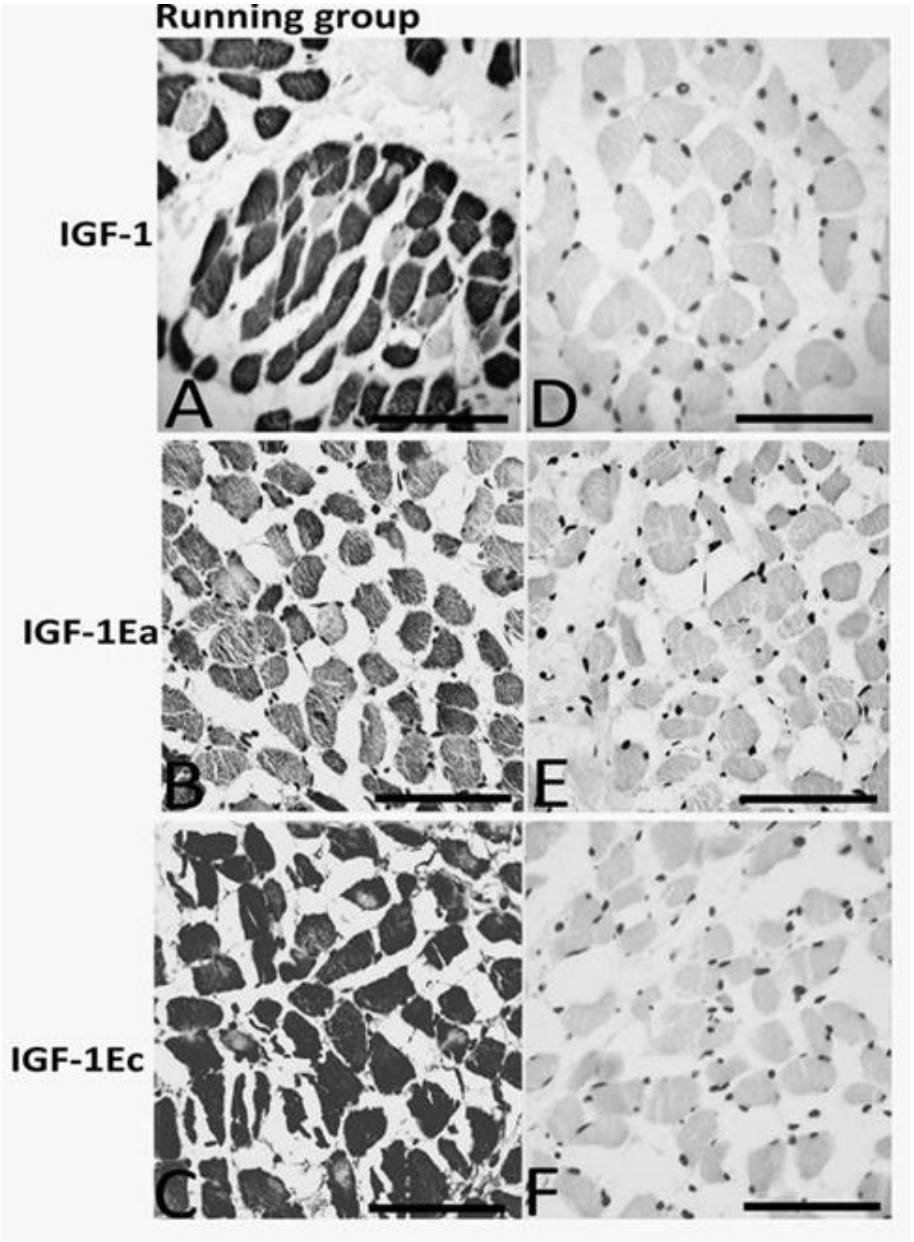


Fig. (4)


Running group
IGF-1
IGF-1Ea
IGF-1Ec
A
B
C
D
E
F

Discussion:

In the present study, serum IGF-1 levels are significantly higher in the runners than that of the sedentary group. This increase in serum IGF-1 was in contrast of Hagberg et al., (1988) who did not find an increase in IGF-1 in long- distance runners after a 60-minutes exer- cise period on the treadmill. In agree- ment with our results, another study de- tected that physical activity increases serum levels of IGF-1 in humans (Carro et al., 2000). Also, Rojas Vega et al., (2010) found that high and low intensity strength exercises increased the circulat- ing levels of IGF-1. More recent study showed significant increase in the serum IGF-1 and cortisol levels by regular aer- obic exercise (treadmill running) in ado- lescents (Jeon and Ha, 2014)

Local and systemic functions of IGF-1 are essential for normal growth and development (Jones and Clemmons, 1995). IGF-1 is produced by a wide range of tissues. About 75% of circulating IGF-1 is derived from the liver (Yakar et al., 1999). It has been shown that exercise stimulates the release of IGF-1 in the liver. However IGF-1 is also expressed locally in other tissues and is supposedly released into the bloodstream to contribute to the other 25% of circulating IGF-1. Exactly which tissues release IGF-1 into the blood- stream is not known, however muscle and fat tissues are the likely candidates (Gatti et al., 2012). In our study, it is possible that the increased levels of se- rum total IGF-I observed in the running individuals could have been a reflection of an upregulation of the IGF-1Ec (MGF) isoform in this group. However the sources of increased levels of serum IGF-1 in runners in this study needs fur-ther investigations.

In our study on the gastrocnemius muscle, IGF-1 is increased on both transcriptional and translational levels by more than 50% and 60% respectively in the running group than that of the seden- tary group. Previous studies reported exercise-induced changes in IGF-I expression, at both the mRNA and protein level (Adams, 1998; Carson et al., 1998; Mozdziak at al., 1998; Goldspink, 1999; Lowe et al.,

1999; Bamman et al., 2001; Hameed et al., 2003). Among those stud- ies which are in agreement with our data, are the previous results on animals which have shown that following muscle activity there was an increase in the ex- pression of IGF-I mRNA in the exercis- ing muscle (De Vol et al., 1990; Czer- winski et al., 1994; Adams and Haddad, 1996, Adams and McCue, 1998). Bam- man et al., (2001) reported a 62 % in- crease in IGF-I mRNA concentration in human muscle 48 h after a single bout of eccentric resistance type exercise.

Expression of IGF-1 gene gives rise to multiple isoforms which differ by signal and termination peptides, and ex- ert different biological functions (Winn et al., 2002; Shavlakadze et al., 2005). Using RT-PCR a single IGF-1 isoform called (IGF-1Ea) could be cloned from the normal resting muscles. However, an additional isoform of IGF-1Eb was de- tected to be expressed in stretched mus- cle undergoing hypertrophy in rabbits (Yang et al., 1996). Another study has shown that resting human skeletal mus- cle expresses at least two isoforms of IGF-I, the systemic liver type which is IGF-IEa and a splice variant, IGF-1Ec or MGF (Yang et al. 1996; Mckoy et al., 1999; Hameed et al., 2003).

In our study, IGF-1Ea levels showed non-significant differences in the serum and in the gastrocnemius IGF- 1Ea mRNA levels between the running and the sedentary group. However, at the protein level IGF-1Ea were higher in the sedentary adolescents than that of the runners. In agreement with our results a previous study found no changes in IGF- IEa mRNA levels in the young human adults practicing resistance exercise ex- periments in their muscles (Hameed et al. 2003). IGF-1Ea is an isoform that is normally expressed in skeletal muscle and was therefore termed mIGF-1 (muscle IGF-1) (Musaro et al. 2001). From our study it seems that IGF-1Ea is not affected in the serum but affected in the muscle activity in adolescents.

The second isoform, which has been classified as IGF-IEb in the rat and IGF-IEc in humans, was first cloned by Yang et al. (1996) in rabbit tibialis ante- rior muscles. IGF-

1Eb mRNA (or MGF in humans) was induced by exercise in rats. Because its expression is mechano-sensitive, it was termed mechano-growth factor (MGF).

In our study there were significant- ly much higher levels of IGF-1Ec in the blood of the runners than that of seden- tary individuals. Also there were signifi- cantly higher levels IGF-1Ec mRNA and IGF-1Ec protein in the running group than that of resting group. These data were in agreement with the previous study that showed a significant increase in IGF-1Ec (MGF) mRNA as a result of exercise. The levels of IGF-1Ec (MGF) were significantly much lower than those of the IGF-IEa isoform in the resting muscles (Hameed et al. 2003). Another study disagree with our results, the study showed that human IGF1Ec mRNA is preferentially upregulated after injury in the skeletal muscle and during the period of muscle remodeling, but not in normal resting or exercising muscles (Philippou et al. 2009).

Increasing the expression of IGF- 1Ea isoform in a mouse has been shown to produce hypertrophic effect on the muscle (Barton-Davies et al. 1998; Mu- saro et al. 2001). Cell culture experi-ments revealed that IGF-1Ea isoforms promoted myogenic differentiation and cell hypertrophy. However, evidence is emerging that IGF-1Ec (MGF) has a different role from that of IGF-1Ea (Yang & Goldspink, 2002) and is very potent (Goldspink and yang, 2001). While IGF-1Ec isoform showed an ef- fect on proliferation of myoblasts in ani- mals (Yang & Goldspink 2002). In our study the differential expression patterns of IGF-1, IGF-1Ea and IGF-1Ec in the sedentary and running groups suggest multiple regulatory systems and multiple roles in those groups. However the roles of IGF-1, and its isoforms in the blood and muscles needs further investigations.

Our data did confirm a different expression profile of IGF-1 and its isoforms both at the mRNA and protein levels in adolescents, which could be consistent with their different physiolog- ical functions on the skeletal muscle in the resting and the running young peo-ple.

Conflict of interests: non

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