EFFECT OF HIGH INTENSITY AEROBIC RUN ON SYSTEMIC BIOAVAILABILITY OF SELECTED MYOKINESAMONG ELITE MIDDLE DISTANCE RUNNERS IN INDIA

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Abstract: Exercise induced myokines secreted by the active muscle tissue during high intensityacute aerobic exercise executes autocrine, paracrine and endocrine cell signalling, intra and inter cellular coordination for homeo-dynamiceffect. For the studyselected myokinesi.e., IL-6, IL-8, IL-15, serum BDNF, Irisin &hormone EPO were investigated through the impact of acute aerobic run.A total of fifteenelite male athletes (n=15)aged21 ± 2.3 years were recruited.The serum samples were collected from ante-medial cubital vein before and immediately after the athletic event, the quantitative assessment of the selected serum myokines and EPO wasdone usingsandwich Enzyme Linked Immunosorbent Assay(ELISA).The resultswere statistically analysed Mean ± Standard Deviation with paired *t*-test at α=0.05 significance.The serum IL-6, IL-8, IL-15, BDNF and Irisin shown significant increasewhere in the p values are <0.01 {IL-6 (before 0.79±0.51 pgm/ml after 5.70±1.59 pgm/ml), IL-8 (before 28.39±10.36 pgm/ml after 61.33±13.63pgm/ml), IL-15 (before 4.58±2.98 pgm/ml after , 8.53± 3.91pgm/ml), serum BDNF (before 14.82±5.86 ngm/ml after 39.01±10.69 nmg/ml)& Irisin (before 89.78±23.82 ngm/ml after 158.88±43.84 ngm/ml)} but serum Erythropoietin levels remained hence the p-value is 0.566(before13.398±4.656 µ IU/ml and after 13.631±5.74 µ IU/ml). The high intensity acute aerobic exercise showed distinct impact on the selected myokines content in the serum levels of the trained elite athletes before and after event but remained the same with EPO.

Key words: IL- Interleukin, BDNF-Brain Derived Neurotropic Factor, EPO- Erythropoietin.

Introduction:

Past two to three decades the focal point with exercise and performance was at molecular level where in the skeletal muscle tissue plays a pivot role in intra and inter tissue metabolism.Molecular exercise endocrinology mainly with skeletal tissue acting as the novel cytokine producer during the exercise, adaptation andperformance[1,2]. The active skeletal muscle tissue producesdifferent cytokinestermed as myokines which have both autocrine and paracrine effects, where in cell itself stimulates in neuronal response there by increasing the cell efficiency and cell stimulates the adjacent cells in response to the rhythmic activity [3].These myokines produce endocrine effect by stimulating other tissues or organs which helpin the intra and inter tissue coordination during acute and chronic cell signalling through intra and inter tissue coordination among different tissues i.e., muscle tissue, adipose tissue, liver, brain,bone etc., for the desired exercise performance[3]. Repeatedbouts of high intensity endurance (aerobic/anaerobic) exercise assists gradual adaptation throughmorphological changes to the conditions at cellular level results change in cellular receptor turnout number, mitochondrial content, myoglobin content, fatty acid oxidation

increased expression of intra cellular m-RNA transcription and protein expression such as IL-2, IL-6, IL-7, IL-8, IL-15, LIF, IL-18, IL-31, TNF-α, Follistatin, Myonectin, Myostatin, Vascular Endothelial Factor etc along with the receptor protein secretion in response to the type of exercise output and neuronal activity [4,5,6,7]. Regular aerobic, anaerobic endurance training increases angiogenesis in the active skeletal muscle tissue [8,9,10]. Individuals performing high intensity endurance activities may be more prone to oxidative stress resulting in physical and physiological adaptation by enhancing antioxidant defence mechanism or increasing oxidative threshold [9,10]. The satellite cells in the skeletal muscle tissue helps the active muscle tissue maintenance, repair and remodelling [11].

Interleukin-6:

Research studies indicate that exercise brings change in the expression of IL-6. The genotypic IL-6 174-G/C polymorphic form has significant impact on the phenotypic expression of IL-6 during exercise, plays a greater role in muscle damage & recoveryin response to eccentric contractions [12], bone mass remodelling [8,14], high density lipoproteins[13,15], glucose tolerance[16]. The genetic polymorphism of IL-6 also plays vital role in the explosive strength of athletes when compared with the endurance athletes[17].

IL-6 was the first to be isolated myokine, having the primary role of IL-6 in anti-inflammatory mechanism. The responses of IL-6 secretion and systemic bioavailability to exercise may depend on the exercise intensity, duration and type including the muscle contraction variety and even the significant systemic increases to many folds did not find complimentary muscle damage[18,19,20,21]. Active muscle enhances the localised and systemic availability of IL-6 thereby promoting both autocrine and paracrine effects in the tissues, causing localised increments in IL-6 receptors expression causing the muscle tissue irritability and triggering the enhanced glucose and fatty acid uptake through the enhanced gp130Rβ/IL-6Rα signalling and activation of AMP kinase/PI3-kinase[5,22,23]. Systemic availability of IL-6 acts as hormone, triggering pathways involving integrating adipose tissue, hepatic tissue, nervous tissue [21,22,23].Intra cellular increase in m-RNA during muscle activity depended on the availability of glucose moiety, lesser the glucose concentrations greater the IL-6 gene transcription leading to increased cytosol m-RNA concentration[23,24,25].The influence of carbohydrate loading has significant effect on the systemic bioavailability of IL-6[26], IL-6 helps in the endogenous glucose production during muscle activity by triggering liver where in the role is mediated along with many other factors [22], like prolonged ingestion of Vitamin-C and Edecreases the release of IL-6 protein (maintaining the transcription of IL-6 m-RNA) from contracting skeletal muscle which decreases systemic bioavailability of IL-6 concentration, there by influences the plasma cortisol concentration[27].

Enhanced plasma concentrations of IL-6 either through exercise or by IL-6 infusion may increase the plasma anti-inflammatory mediators like IL-1ra & IL-10, cortisol[28,29,30]and alsocould cause for enhanced circulatory neutrophils without corresponding increases in the plasma epinephrine levels and causes for suppression of TNF-α[31]. Hence, theexercise induced or systemic infusion of IL-6 may help protection from the TNF-α mediated insulin resistance [32].

The localized effect of exercise induced IL-6 plays an important role in the regulation of satellite cell (muscle stem cell)mediated hypertrophic muscle growth [33]. On summary,

exercise induces IL-6 expression by the active muscle tissue plays a major role in autocrine and paracrine mechanism, secreted into the plasma plays a major role as endocrine stimulant causes inter tissue coordination, anti-inflammatoryand metabolic effects like fatty acid oxidation, cortisol mediated insulin resistance etc.

Interleukin-8:

Research studies indicate that the exercise changes in expression of IL-8 remained complex. Acute bout of aerobic exercise significantly enhances the localised IL-8 m-RNA and protein levels in active muscle tissue, but the plasma IL-8 concentrationsremained precarious [24]. High Intensity Interval Exercises,Marathon runs and eccentric muscular concentration shown significantelevationof plasma IL-8 concentrations [34,35,36,37,38]contrary moderate intensity run's, concentric contraction {bicycle ergometry and rowing}plasma IL-8 concentrations remained almostbaseline level[34,39,40].

The pre exercise muscle glucose loading has similar effect with IL-6 m-RNA secretions, low glucose muscle loading pre-exercise increases localised IL-6 and IL-8 m-RNA levels, but systemic protein concentrations IL-6 and IL-8 differed significantly as IL-6 levels increases but IL-8 levels remained unchanged[38,40].

IL-8 belongs to CXC chemokinetic subfamily containing Glu-Leu-Arg(GLR motif). Post hour endurance exercise increases CXCR-1 and CXCR-2 receptors m-RNA and receptor protein expression in active muscle tissue [7,42]. The CXC chemokines with GLR motifs exhibit potential angiogenic effectwith high receptor bonding affinity[7,41,42,43], lack of GLR motif act as inhibitors of angiogenesis[42]. The binding of IL-8 to the CXCR-1 and CXCR-2 receptors plays vital role in the angiogenetic[44,45] effect along with the vascular endothelial growth factor[46,47] and Transforming growth factor-β[48]by chemotactic response through CXCR-1 receptor.

Interleukin-15 (IL-15):

The role of exercise induced Interleukin-15expression at localised and systemic effect remains controversial. The resting plasma Interleukin-15 level remains elevated in lean and obese - non active subjects on par with active subjects [55]. Exercise trainingprotocolsmay havechanged effect on IL-15 gene expression,IL-15 m-RNA and IL-15 protein levelsin active skeletal muscle tissue.Resistance training on active skeletal muscle tissues with type-II muscle fibres produce greaterIL-15 m-RNAwith Type-I muscle tissue [49,50,51], but plasma IL-15 expression remains controversial, research findingsindicate that the plasma IL-15 level doesn't change in chronic resistance activitybut there is an increase in the localised IL-15 protein expression[49,50,52]. Aerobic endurance exercise had similar m-RNA levels compared with the resistance exercise, but the plasma protein content varies based onduration of activity 30min, 2hrs and 3hrs[50,51,52,53,54].

The localised IL-15 protein expression in active muscle tissue prevents skeletal muscle tissue protein degradation[56,57,58], musclenuclear apoptosis[56], increased glucose uptakethrough Jak3/STAT3 [59],fatty acid oxidation [57] mitochondrial activity and biogenesis[56,60].

IL-15 Myokine secreted during muscle activity in the systemic circulation plays major role in muscle and adipose tissue coordination [56,57,60]. Localised and systemic IL-15 plays major role in the reduction of adipose tissue through activation of peroxisomal proliferator activated

receptor [61]. Increase in plasma IL-15 protein expression helps in decrease of lipid uptake and also helps in lipid metabolism [60,62]. Thus, exercise induced IL-15 plays a significant role in muscle fat cross talk.

Serum BDNF:

Research studies in past decade on Brain Derived Neurotropic Factor (BDNF)and its effects received greater attention as it plays vital role in brain related disorders and recovery. The role of BDNF remains complex as it belongs to the family of neurotrophins, helps in neurogenesis, retaining or maintaining synapsis[63] throughNeuro plasticity[64]& cognitive role for effective memory[65,66] and plays vital role in metabotropins through energy homeostasis, glucose and fatty acid metabolism[67,68]. BDNF produced in the brain or peripheral tissues can cross either side of blood brain barrier through high capacity saturable system [69].

BDNF plays an important role in diet intake, research findings by Yamanaka at al., 2007 and Suva et al., 2010 increased serum BDNF or subcutaneous BDNF administration significantly reduced food intake and increase glucose uptake byincreasing glucose transporter 4 expression in the skeletal muscle tissue [72,73,74]. Regular exercise will enhance BDNF levels in the skeletal muscle tissue which helps in fat oxidation by activation of AMP Kinase [74].

Regularacute bout of resistance or endurance exercise will enhance the circulating[70,71,72],hippocampal BDNF [72,73] and skeletal muscle tissue m-RNA levels[74]. Regular trained athletes or habitual exercise showed distinctly lesser circulating BDNF than sedentary persons [75,76]. Exercise increases BDNF m-RNA and BDNF protein levels,majority of the localised muscle BDNF levels doesn't show into the systemic asit acts locally through autocrine and paracrine mechanism[74,77]. About 70 to 80% of peripheral protein BDNF levels brain is the primary source in response to physical activity [78].

Irisin:

Irisin is a novel polypeptide (discovered in 2012) cytokine produced by the active skeletal muscle tissue which is secreted into systemic circulation following high intensity exerciseplays vital role in metabolism and energy homeostasis [81,82,83,84].FNDC-5 m-RNA levels increases in the active muscle tissue after a single bout of exercise [87].Irisin released into the systemic circulation after the cleavage of membrane protein Fibronectin Type-III Domain Containing Protein-5 (FNDC-5) predominantly expressed by the active muscle tissueafter single bout of aerobic endurance exercise and resistance exercise[84].The expression of myokine (FNDC-5 precursor of Irisin) in the skeletal muscle tissue is predominantly under control of Transcriptional Co-activator PPAR-γ coactivator-1α (PGC1-α) which plays a major role in the physiological adaptations in endurance training andmitochondrial biosynthesis [85,86]. The systemic protein irisin acts on adipose tissue by enhancing thermogenesis of brown adipose tissue from white adipose tissue through expression of Uncoupling protein-1 {UCP-1}[85].

Irisin plays major role in reversing insulin resistance, as insulin resistance in the muscle tissue acts by blocking glucose transporter-4 (GLUT-4) where in the circulating levels of glucose moieties increases [82,85].The elevated localised and systemic irisin levels during active muscle tissuehelps in retaining glucose uptake and mitochondrial activity through

p38/MAPK-PGC1-α [82,85].Approximately 72% ofcirculating Irisin levels accounts for the muscle and remaining 28% accounts for adipose tissue, heart, rectum and tongue[85,88].

Central and systemic of Irisin shows anti-depressant effects by stimulating the genes related to enhance neuroplasticity in cortex and hippocampal region in mice[89], protect against neuronal injury recovery by activation of AKT and ERK1/2 pathways[90]. Irisin also plays vital role as therapeutic agent in metabolic related diseases such as non-alcoholic fatty liver (NAFLD), Insulin Resistance (IR), [82,85,90,91].

Erythropoietin:

Exercise induces increased expression of Erythropoietin receptor (EPOR) m-RNA levels in active skeletal muscle satellite cells [12]. The expressed EPOR m-RNA doesn't account to the protein synthesis and cellular expression [92,93].Erythropoietin plays vital role in erythropoiesis by acting on pluripotent cells of bone marrow for erythrocyte proliferation and differentiation [94,95].

Methods:

A total of fifteen male (n=15) middle distance elite athletes, aged between 21±2.3 years were recruited for the study. The runners were reported for not having any acute and chronic pathological conditions for 6 months prior to the conduct of the study. The athletes were observed to be in the specific discipline of their training for at least 5 years before shortlisting.The athletes were reported to be medallists or finalists at national tournaments representing either their respective states or universities in India. The athletes were asked to give their best timing during the 3000 mts race.

Blood Sampling:

5ml of Blood samples were collected form the athletes before and immediately after the commencement of specified middle distance run from ante medial cubital vein into the colt activator serum separating tubes(purchased from Levram Lifesciences Pvt. Ltd). The collected blood was positioned ideally for 5 min at room temperature for serum separation, werein the tubes were centrifuged at 2000 rpm for 10 minutes for clear serum (Remi R-8C centrifuge). Thus, obtained clear serum was separated and collected into 2ml Eppendorf safe lock tubes (Tarson). These Eppendorf tubes were stored at -20^0C for transportation to the researcher's lab where they were frozen at -80^0C tillthe conduct of biochemical estimates.

Serum Myokine Measurement:

The levels of myokine estimate in the serum samples were analysed by usingcommercially available quantitative sandwich human ELISA kits from BiBiotech India (IL-6, IL-8, IL-15, EPO and Irisin) (R&D systems, USA), Yannic Life Sciences (BDNF) (Biolegend, USA) and Duo set ancillary Reagent Kit-2 procured from Bi Biotech India (R&D systems, USA). The uncoated flat bottomed microplates were pre-coated with specified capture antibody a day before the analysis of the serum samples. The procedure and the analysis were adopted in accordance with the standard operating procedure specified in the certificate of analysis and Duo set ELISA development system (catalogue number DY: 206-05)provided by the manufacturer. The samples optical density was measured using Thermo scientific multiskan EX (Finland)with Ascent software Version 2.6 at 450nm where in the corrections were made at 570nm in each run plate.

Ethics Approval:

All the procedures performed under the study was in accordance with the Ethical Standards laid down by the Institutional Human Ethical committee.The research protocol was approved by the Institutional Ethics Committee for Biomedical Research (Institute of Genetics and Hospital for Genetic Diseases O.No: 28/IEC/IOG/OU/18 dated: 05-02-2018). The athletes were given information about theresearch work and testing protocols where in after their voluntary willingness of participation, the written informed consent form the athletes were obtained prior to the study.

Statistical Analysis:

The statistical analysis of the results was analysed based on SPSS software (version 20.0: SPSS Inc, Chicago, Illinois, USA). The data was presented on Mean ± Standard Deviation. The results were statistically analysed based on paired *t*-test where in the level of significance was fixed at 0.05.

Results:

After running the race protocol designed by the researcher, the athletes who have volunteered to participate in the study had ran 3000mts with 538 ± 52 seconds where in the samples were analysed through sandwich ELISA test the quantitative analysis of the serum samples were presented in the table.

	Mean	**Std Dev.**	**df**	**t-value**	**p-value**
Pre IL-6 (pico gm/ml)	0.80	0.51	14	9.650	<0.01
Post IL-6 (pico gm/ml)	5.43	1.815			
Pre IL-8 (pico gm/ml)	28.39	10.36	14	7.548	<0.01
Post IL-8 (pico gm/ml)	61.33	13.63			
Pre IL-15 (pico gm/ml)	4.58	2.98	14	9.851	<0.01
Post IL-15 (pico gm/ml)	8.53	3.91			
Pre BDNF (ng/ml)	14.82	5.86	14	8.049	<0.01
Post BDNF (ng/ml)	39.01	10.69			
Pre Irisin (ng/ml)	89.78	23.82	14	5.922	<0.01
Post Irisin (ng/ml)	158.88	43.84			
Pre EPO (µI/ml)	13.398	4.656	14	0.588	0.566
Post EPO (µI/ml)	13.631	5.74			

Table1: paired t-test statistics of serum IL-6, IL-8, IL-15, BDNF, Irisin and EPO (α=0.05; t-critical = 2.145)

Therewas a significant raise in the average serum IL-6, IL-8, IL-15, serum BDNF and Irisin content before the commencement of event 0.79±0.51 pgm/ml, 28.39±10.36 pgm/ml, 4.58±2.98 pgm/ml, 14.82±5.86 ngm/ml&89.78±23.82 ngm/ml and immediatelyafter completion of event5.70±1.59 pgm/ml, 61.33±13.63pgm/ml, 8.53± 3.91pgm/ml, 39.01±10.69 nmg/ml&158.88±43.84 ngm/ml.A significant impact was observed when statistical student's paired *t*-test was appliedwith t value's 10.969, 7.54, 9.85, 8.05, 5.92was found to be greater than the table value of 2.145 shown in the table1.

The serum EPO concentrations almost remained the same before13.398±4.656 µ IU/ml and after 13.631±5.74 µ IU/ml with student's paired t-test value of 0.588 (p value of 0.566) less than the table value 2.145

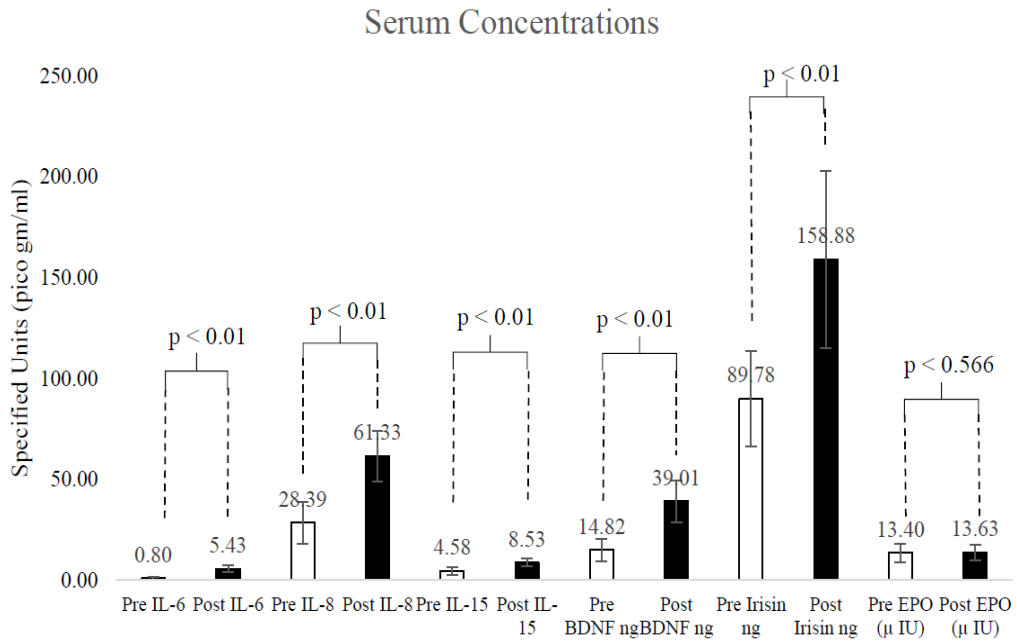

Fig1: serum concentrations on acute bout of aerobic run of IL-6, IL-8, IL-15, BDNF, Irisin and EPO (IL-6, IL-8, IL-15 – units of measurement pico gm/ml)

Discussion:

The present study examined the influence of acute bout of high intensity aerobic run among elite middle distance runners on the expression of the selected myokine expression in the serum levels implies there is significant rise of serum myokines throughpost event. These changes also inferred throughprevious research's [28,29,55] where in there is a sharp and significant rise in IL-6, IL-8, IL-15, serum BDNF and Irisin myokine levels in the serum levelswas observed with individual post event. In contrast there is no significant increase in the systemic levels of serum EPO concentrations post event (Fig: 1).

High intensity acute bout of aerobic run had shown increase in the serum concentrations of IL-6, IL-8, IL-15, BDNF, LIF and TNF-α [39,55,96].Skeletal muscle injury also increases the production of localised IL-6 m-RNA levels and IL-6 protein levels through

the increased stimulation ofIL-1β and TNF-α [28].IL-6 also prevents the secretion of IL-1β by triggering monocytes for the production of IL-1ra which serves as negative feedback mechanism for increased localised inflammatory response [39,96]. High intensity aerobic event triggers increased systemic levels of IL-6 post running and cycling[21,33,96]. IL-8 plays major role in the chemokinetic response by mobilization of leucocytes [34]. Exercise induces the localised active skeletal muscle tissue IL-8 m-RNA and protein synthesis which plays vital role in autocrine and paracrine mechanism by inducing angiogenesis and also plays major role in increase of localised leucocyte number [7].IL-15 protein expression also increases in systemic level after endurance run with similar findings in Tamura et al., 2011. Increased systemic levels of IL-15 also plays a vital role with angiogenesis as endocrine effect[42,43]. Over expression of IL-15 in transgenic mice Quinn et al., 2011plays a major role in muscular adaptation, metabolic adaptation and muscle hypertrophy in response to exercise [55].

The study had demonstrated that acute bout of high intensity aerobic run had significantly increased the systemic levels of BDNF on similar lines with many researches Schmidt-Kassow et al., 2012, Mathews et al., 2009.The secretion also depends on the nature and intensity of exercise (acute, chronic, low intensity and high intensity)[97]. Chronic high intensity endurance training showed lower serum BDNF concentrations with nominal subjects [75,76]. Exercise induces increased expression of FNDC-5 (precursor of Irisin) in active skeletal muscle tissue[85].

Conclusion:

The results shown the distinct impact of exercise on serum myokines and hormone erythropoietin levelsthrough single acute bout of aerobic run designed by the researcher. There is a significant increase in the serum IL-6, IL-8, IL-15, BDNF and Irisin post run after the athletes progressed through run, but serum EPO concentrations remained the same.

Ethics Statement:

The protocol and study were conducted based on the guidelines laid by the “Institutional Ethical Committee for Biomedical Research of Osmania University”with written consent form submitted by the athletes. All the athletes gave their informed consent form in accordance to the declaration of ICMR.

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