Impact of Acute Exercise by Yo-Yo Intermittent Recovery Test on Hematopoietic Stem Cells, Muscle Damage and Inflammations Markers in Football Players

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Abstract

Introduction: Circulating stem cells can reach various organs and become cells of that organ, helping such organ regain and maintain optimal health. Purpose: The effect of level-1 Yo-Yo Intermittent Recovery Test on Hematopoietic stem cells, Muscle Damage and inflammations Markers in Football Players. Methods: Fifteen healthy male's athlete's (especially football players from second division clubs) performed Yo-Yo Intermittent Recovery Test Level 1 age (21.4± 1.96 y). The Yo-Yo IR1 test involves running between two markers 20 meters apart. The participants have an active break of 10 seconds before running 40 meters again at regular intervals, the required running speed increases. The test continues until the participants are no longer able to keep up with the required pace. Blood samples taken pre, and immediately after Yo-Yo IR1 test were analyzed for hematopoietic stem cells (HSCs), creatinine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), lactic acid (LA), C-reactive protein (CRP) and white blood cells count (WBC). HSCs were quantified by flow cytometry. CK, LDH, AST, LA, CRP and WBC analysis were obtained by standard methods. Results: Immediately after The Yo-Yo IR1 test there was significant increase in HSCs, CK, LDH, AST, LA, CRP and WBC p < 0.05.. Conclusion: These data indicate that The Yo-Yo IR1 test increased circulating hematopoietic stem cells, muscle damage in the blood stream differently in football players.

Key words: stem cells, hematopoietic stem cells, muscle damage, Yo-Yo IR1 test.

Introduction and Research Problem

Growth, regeneration, and repair of tissues are dependent on the addition of new differentiated cells. These cells are mainly derived from undifferentiated cells which can proliferate and differentiate. These cells are called "stem cells" or "progenitor cells." These cells are sources for tissue regeneration and repair as well as tissue growth (Beausejour et al., 2007). These stem cells are released from bone marrow to peripheral blood in response to certain stimuli (exercise, injuries) and guided by gradients of growth factors and cytokines (released by the damaged or loaded tissue) to the sites where they are needed and migrate into various tissues and organs. These bone-marrow-derived stem cells might support organ-resident stem cells or perform different functions for the regeneration and adaptation of the tissue.

A major source of these circulating stem and progenitor cells is the bone marrow, with its different stem and progenitor cell populations, such as hematopoietic stem cells (HSCs) (Ratajczak et al., 2007; Wu Y et al., 2007). HSCs were first discovered in 1961 as the donor cell population responsible for survival and regeneration of all blood cell lineages in myeloablated hosts (Till and McCulloch EA 1961). HSCs are the most primitive cells in the hematopoietic lineage and act as a reserve cell population responsible for maintenance and production of circulating blood cells (Metcalf D.2007).

The results indicated that, besides their normal function of regulating blood cells, HSCs (such as CD45+:Sca1+ cells) are also involved in skeletal muscle regeneration (Mori et al., 2008). Dormant HSCs are also located in niches at the endosteum, whereas activated HSCs are in close contact with sinusoids of the bone marrow microvasculature (Wilson et al., 2007). A number of studies have reported changes in circulating hematopoietic stem cells. For example (Zaldivar et al., 2007) shown that exercise (20 min of moderate-to-vigorous cycle ergometer exercise) can affect HSC and HSC mediators (SDF-1, granulocyte colony-stimulating factor). Morici et al reported that Supramaximal exercise doubled the number of circulating CD34+ cells (Morici et al 2005). Thijssen et al investigated the effects of training and aging on HSCs (Thijssen et al., 2006). Acute exercise significantly

increased the number of HSCs. Older men showed significantly lower baseline and exercise-induced levels of HSCs than young men. Therefore, it seems that advancing age results in lower circulating numbers of HSCs and attenuates the acute-exercise-induced increase in the number of HSCs. Exercise provokes a number of stimuli: mechanical, metabolic and hypoxic. It also induces the release of various growth factors, cytokines and hormones (Laufs *et al.*, 2004). Circulating hematopoietic stem cells and muscle damage are well documented in response to aerobic exercise. However, far less information is available on level-1 Yo-Yo Intermittent Recovery Test (The Yo-Yo IR1 test) in football player's induced hematopoietic stem cells and muscle damage.

Aim of the Research

The researchers aim to identify the effect of level-1 Yo-Yo Intermittent Recovery Test (The Yo-Yo IR1 test) on the changes of HSCs, muscle damage and inflammations markers.in football players.

Research questions

1- What is the effect of level-1 Yo-Yo Intermittent Recovery Test (The Yo-Yo IR1 test) on the changes of HSCs in football players?

2- What is the effect of level-1 Yo-Yo Intermittent Recovery Test (The Yo-Yo IR1 test) on the changes of muscle damage and inflammations markers in football players?

Research Procedures

Due to the nature of this study, researchers have used experiments attitude to the measuring before and after exercise on one experimental research groups.

Sample of research:

Researchers have chosen the research sample at purpose method one groups from Egyptians players ages (21.4 ± 1.96) y with training age from 7y to 10y.

Collection data method

Researchers have used group means and tools of collecting data also use some equipment's and tools which help the researcher in making the search such as the following:

- References and literature.

Form collect and record data players. -

Physical measurements and tests (Yo-Yo Intermittent Recovery Test 1)-

Devices and tools used in the search

- Monoclonal antibodies against human (CD34pos/CD45pos).

- Fluorochrome – conjugated antibodies.

- Automatic Mindray 3200 Hematology Analyzer.

- Centrifuge **4000 rpm.**

- EDTA anticoagulant

- Stopwatch

- Polar Watch

- Human body composition monitoring device

- Audio cues of Yo-Yo IR1 test (CD player)

Research Domains

Research Time and Place

This study was conducted from 20Jan to 20 March 2020 in the following places:

- Faculty of Physical Education Mansoura University

- Regenerative Medicine Center Mansoura University

- Alsahaba Medical Laboratory in Mansoura

- Smart Lab Laboratory in Mansoura

Methods

Participants and Study Design

Fifteen healthy male's athletes' (especially football from second division clubs Mansoura - Mahala - Dekerness - Talkha age 21.4 ± 1.96 years) participated in this study. Subjects did not use any medication 8 weeks before the study until the end. The study was approved by the local Ethics Committee, and all participants gave their written informed consent. Subjects had no medical history, no vascular risk factors and had no medical treatment. Physical examination and venous blood sampling excluded apparent pathologies (for physical characteristics of the subjects see Table 1).

Exercise protocol

Fifteen healthy male's athlete's (football players) performed Yo-Yo Intermittent Recovery Test Level 1 (The Yo-Yo IR1 test). The Yo-Yo IR1 test involves running between two markers 20 meters apart, following audio cues (CD) (which dictate the running speed required. After each 40 meters run, the participants have an active break of 10 seconds before running 40 meters again. At regular intervals, the required running speed increases. The test

continues until the participants are no longer able to keep up with the required pace. VO^2 Max and the total distance (TD) covered during the YYIRT level 1 was considered as the test score Table (2). The Yo-Yo IR1 test (was performed according to the procedures suggested by Krustrup et al and Bangsbo et al; test reliability was established in a previous studies (Krustrup et al., 2003, Bangsbo et al.,2008).

Blood sampling and analysis

After an overnight fast from 10h to 12h blood samples (Venous blood) taken pre, and immediately after exercise were analyzed for hematopoietic stem cells (HSCs / CD34pos/CD45pos) , creatine kinase (CK) , lactate dehydrogenase (LDH), aspartate aminotransferase (AST), lactic acid (LA), and white blood cells count (WBC) . HSCs were quantified by flow cytometry. CK, LDH, AST, LA and WBC analysis were obtained by standard methods. The concentration of C-reactive protein (CRP) in the serum was measured using a turbidity assay (Elitech Clinical System- France). Low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, cholesterol, SGOT and Creatinine concentrations were measured by

enzymatic in situ assays with photometric final quantification (Roche Diagnostics).Complete blood counts (CBC) for white blood cell analysis, Serum CK, LDH, AST and LA, were obtained by standard methods from the clinical hematology laboratory at MERC and Alsahaba Medical Laboratory.

Flow cytometry analysis

Reagents

The following antibodies were used: fluorescein isothiocyanate (FITC)– labeled anti-CD،٣٤ ECD-labeled anti-CD 45, both purchased from immunotech, Beckman Coulter, Marseille, France.

Cellular immunophenotype analysis:

After incubation of blood sample with a mixture of fluorescence labeled anti-CD34, anti-CD45 for 15 minutes, lysis of red blood cells was done using 10Test 3 lysis solution (Immunotech, Beckman Coulter, and Marseille, France). Analysis was done using the EPICS XL flow cytometer (Coulter Electronic, Fl, USA). After gating on $CD45^+$ we analyzed for expression of. At least 10000 cells per sample were analyzed.

Statistical analysis

All values were presented as mean and standard deviation (SD). Data were analyzed using Paired – Samples T Test to examine the difference between before and after Yo-Yo Intermittent Recovery Test 1. The level of significance was set at $p < 0.05$. All analysis was conducted with SPSS software (version 22.0).

Results

Table (1) Anthropometric and physiological Characteristics

N= 15	Mean	SD
Age (years)	21.4	1.96
Height (cm)	173.55	5.25
Weight (kg)	71.04	5.97
Rest Hear Rate (RHR)	61.7	1.5
Body mass index, kg/m2	23.48	1.46
Systolic blood pressure (mmHg)	122.5	3.63
Diastolic blood pressure (mmHg)	80.1	1.20
Cholesterol, mg/l	157.7	8.55
Triglyceride, mg/l	50.2	6.05
HDL, mg/l	60.88	3.98
LDL, mg/l	90.05	5.28
SGOT, U/l	23.4	1.84
Creatinine (mg/dl)	0.74	0.16

Data presented as mean ± standard deviation. HDL, high-density lipoprotein; LDL, low-density lipoprotein; SGOT, Serum glutamic oxaloacetic transaminase.

Table (2) Yo-Yo Intermittent Recovery Test 1 results (N = 15)

Variables	Mean	SD
Total Distance Run (meters)	1980	122.57
Total Time (mins)	16.26	6.92
VO2 max (ml/kg/min)	53.04	1.02

Data presented as mean ± standard deviation.

Table (3)

Changes in Muscle Damage Markers assessed by Serum Creatine kinase (CK), Lactate dehydrogenase (LDH) and Aspartate aminotransferase (AST) before and immediately (after) Yo-Yo Intermittent Recovery Test 1. (N = 15)

Parameter	before Yo-Yo IR1 Mean (SD)	after Yo-Yo IR1 Mean (SD)	Rate of Change %	t	P-Value
LDH (IU/L)	345.9 ± 19.86	414.8 ± 41.22[*]	19.92 %	6.00	.001
CK (IU/L)	176.5 ± 36.10	237.9 ± 44.93[*]	35.79%	9.60	.001
AST (U/l)	24.69 ± 3.20	36.70 ± 4.90[*]	48.64%	6.82	.001

*A significant level P<0.05. ; Data presented as mean ± standard deviation.

Table (3): LDH, CK and AST increases significantly immediately after Yo-Yo IR1 .

Table (4)

Changes in Inflammations and Muscles Fatigue Markers assessed by Serum Lactate, White Blood Cells (WBCs) and C-reactive protein (CRP) before and immediately (after) Yo-Yo Intermittent Recovery Test 1. (N = 15)

Parameter	before Yo-Yo IR1 Mean (SD)	after Yo-Yo IR1 Mean (SD)	Rate of Change %	t	P-Value
Lactate (mmol/l)	1.39 ± 0.25	5.96 ± 1.04[*]	328.78%	12.691	.001
WBCs (Gpt/l)	5.84 ± 0.97	8.33 ± 1.57[*]	42.64%	6.50	.001
CRP (mg/l)	0.72 ± 0.29	1.07 ± 0.41[*]	48.61%	2.49	.034

*A significant level P<0.05. ; Data presented as mean ± standard deviation.

Table (4): Lactate, WBCs and CRP increases significantly immediately after Yo-Yo IR1.

Table (5)

Changes in Hematopoietic stem cells (HSCs) (CD34pos/CD45pos) before and immediately (after) Yo-Yo Intermittent Recovery Test 1. (N = 15)

Parameter	before Yo-Yo IR1 Mean (SD)	after Yo-Yo IR1 Mean (SD)	Rate of Change %	t	P-Value
HSCs (CD34pos/CD45pos) (cells/ml)	0.078 ± .016	0.111 ± 0.017[*]	42.31%	6.98	.001

*A significant level P<0.05. ; Data presented as mean ± standard deviation.

Table (5): Hematopoietic stem cells (HSCs) (CD34pos/CD45pos) increased significantly immediately after Yo-Yo IR1 amounted to ($_{0.111 \pm 0.016}$ ml) (p < 0.05) in addition this increased represents a percent $_{42.31\%}$in the peripheral blood compared with measurements before Yo-Yo IR1.

Discussion

The main goal of this study was to know the effect of exercise bout (level-1 Yo-Yo Intermittent Recovery Test) on changes of hematopoietic stem cells and muscle damage in football players. Far less studies are available in the literature on the effects of (level-1 Yo-Yo Intermittent Recovery Test) on HSCs, defined as CD34pos/CD45pos.

Limited studies have reported changes in circulating HSCs following aerobic and anaerobic exercise. For example (Morici et al., 2005) reported that supramaximal rowing exercise (1000 m) doubled the number of circulating HSCs in young, well-trained athletes. In a more recent study, the post exercise changes in several bone marrow-derived hemopoietic and angiogenic progenitors were compared between a marathon and a 1500-m run, and (Bonsignore et al., 2010) reported

that HSCs did not change after a marathon, but increased after a 1500-m run. Acute exercise significantly increased the number of HSCs. Older men showed significantly lower baseline and exercise-induced levels of HSCs than young men (Thijssen et al., 2006). Mohammed Nader Shalaby et al. (2017) reported that CD34+ HPC counts were increased in peripheral blood of anaerobic exercise bout than aerobic one due to stress induced by anaerobic exercise bout.

Three important messages emerge from this study. First, performing level-1 Yo-Yo Intermittent Recovery Test triggers an inflammatory response and muscle fatigue as evident by a significant rise in WBCs count, CRP in addition to LA immediately after the Yo-Yo IR1 test. Second, muscle damage markers (CK, LDH and AST) increased significantly Yo-Yo IR1 test. Third, immediately after the Yo-Yo IR1 test the Percent of circulating HSCs, defined as CD34pos/CD45pos was significantly increased. Taken together, these results suggest that Yo-Yo IR1 test leads to an inflammatory response, muscle fatigue immediately after exercise and increase in muscle damage in in addition to

increases of circulating hematopoietic stem cells.

No previous studies have investigated changes in hematopoietic stem cells following Yo-Yo IR1 test. Therefore, the researcher believes that the increased in HSCs immediately after Yo-Yo IR1 test may be the result of the endurance performance of Yo-Yo IR1 test that caused muscle fatigue and inflammation, as evident by significant increase in the concentration of LA and WBCs count in addition to significant increase in muscle damage markers immediately test. So the percent of HSCs was increased in peripheral blood due to its migration from the peripheral blood to organs to repair and regenerate the damaged tissue. It is likely that HSCs can migrate into skeletal muscle and constitute, at least in part, a multipotent muscle-derived stem cell population (Burdzinska et al., 2008). In the present study HSCs were increased significantly in after Yo-Yo IR1 test in the peripheral blood compared with measurements before exercise.

HSCs are released from bone marrow to peripheral blood in response to certain stimuli (exercise, injuries). These stem cells are guided by gradients of growth factors and cytokines (released by the damaged or loaded tissue) to the sites where they are needed and migrate into various tissues and organs (*Rojas*, *Mauricio* 2010). In this study, the activity of CK and AST enzyme levels increased significantly immediately after Yo-Yo IR1 test compared with measurements before exercise, in addition LDH enzyme level increased significantly in the immediately after exe Yo-Yo IR1 test compared with measurements before exercise. The enzymes special to muscle cells are used for determination of muscle damage. These enzymes are: creatinine kinase (CK), aspartate amino transferase (AST) and lactate dehydrogenase (LDH) (Skenderi et al., 2006; Jiang et al., 1998). When muscle damage occurs, damage marker increases in the plasma after the exercise. CK, AST and LDH are usually considered as muscle damage markers (Wilson et al., 2008; Kim et al., 2007; Clarkson et al., 1986; Smith and Miles, 2000). The researchers believe that increase in HSCs in the immediately after Yo-Yo IR1 test explains the need of damaged tissues and muscles to more HSCs as a result of muscle damage which occurs after Yo-Yo IR1 test, as evident by significant

increase in CK, LDH and AST for the ability of HSCs to participate in muscle regeneration. The ability of mobilized stem cells to home to areas of injury and participate in tissue repair has been documented, and as such, these mobilized cells may serve as part of a repair mechanism after exercise or injury (.Marycz Ket al., 2016; Adam W. Anz, et al., 2019). Other results indicated that, besides their normal function of regulating blood cells, HSCs (such as CD45+:Sca1+ cells) are also involved in skeletal muscle regeneration (Mori et al., 2008).

Conclusion

This study demonstrates that Yo-Yo IR1 test -induced muscle damage markers, inflammation leads to a significant increase in HSCs immediately after exercise to involve in skeletal muscle regeneration besides their normal function of regulating blood cells. Further studies are necessary to investigate the role of circulating progenitor cells in muscle regeneration after Yo-Yo IR1 test in football players.

Recommendations: -

1- Carry out similar studies on different age levels in both sexes (males - females) on large samples and different physical loads on athletes, practitioners and non-athletes for large time periods and long-term training programs for the study of molecular adaptations that occur to stem cells at the level of (human - experimental animals).

2- The need for the use of experimental animals in the future scientific research in the field of stem cells and sport to benefit from taking samples of tissues of human and experimental animals in addition to the blood samples to try to understand the changes that occur at the level of tissue and blood.

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تأثير التمرين الحاد بواسطة المستوى الأول لأختبار يويو المتقطع الراحة على الخلايا الجذعية المنشئة للدم و علامات التلف العضلى والالتهابات لدى لاعبى كرة القدم

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مقدمة : تستطيع الخلايا الجذعية أن تصل الى العديد من الأعضاء عبر الدورة الدموية وتصبح جزء من خلايا هذه الاعضاء ومساعدتها على التجدد وإستعادة مستوى صحى مثالى . الهدف : من هذه الدراسة هو التعرف على تأثير المستوى الأول لأختبار يويو المتقطع الراحة (Yo-Yo IR1 test)على الخلايا الجذعية المكونة للدم وعلامات التلف العضلى والالتهابات لدى لاعبى كرة القدم . الطرق : عدد (15 رياضيين ذكور إصحاء) من لاعبى الدرجة التانيه المصريين لكرة القدم وبلغ متوسط أعمارهم السنية (y 21.4± 1.96) أدوا إختبار Yo-Yo IR1 test المكون من (٢ × ٢٠ متر) جرى ذهاب وعودة يتخللهم ١٠ث للراحة بين كل تكرار حتى الوصول للإجهاد وعدم المقدرة على التكرار. تم أخذ عينات الدم قبل وبعد المجهود مباشرة للتعرف على إستجابات المتغيرات الخلايا الجذعية المكونة للدم (HSC) ، إنزيم الكرياتين كينيز (CK) ، إنزيم اللاكتات دى هيدروجيناز (LDH) ، إنزيم أسبرتيت أمينوترانس فيريز (AST) ، حمض اللاكتيك (LA) ، كرات الدم البيضاء (WBCs) . تم تحليل الخلايا الجذعية المكونة للدم بواسطة جهاز التدفق الخلوى Flow cytometry . النتائج : بعد أداء إختبار Yo-Yo IR1 test وجد زياده دالة إحصائيا فى المتغيرات WBC – CRP– LA -AST - LDH - CK - HSCs عند مستوى معنوية 0.05 > لصالح القياس البعدي . الخاتمة هذه البيانات تشير إلى ان إختبار Yo-Yo IR1 test زاد من من إستجابات الخلايا الجذعية المكونة للدم وعلامات التلف العضلى والالتهابات بالدورة الدموية والدم الطرفى بنتائج مختلفة بعد الاختبار مباشرة .

مفتاح الكلمات: الخلايا الجذعية ، الخلايا الجذعية المنشئة للدم ، التلف العضلى ، المستوى الأول لأختبار يويو المتقطع الراحة