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Fat Oxidation Substrates Responses to Heparin Injection during Cycling in Male Students

Short Running title
Fat oxidation in relation to heparin injection

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ABSTRACT

Background and Objective: This study was performed to determine whether heparin injection affects some physiological and metabolic marker during exercise in none-trained boy students. **Methods**: For this purpose, forty none-trained boy students aged 18 – 24 years were divided to experimental (heparin injection) a control (placebo injection) groups randomly. At first all participant of two groups performed a cycling ergometery test according to Astrand submaximal protocol without heparin or lactose injection. After 7 days, they repeated this protocol while heparin (experimental) or lactose (control) injection 30 min before exercise test. Immediately after each test, blood sampling was taken for measuring of lipid metabolism determinants such as free fatty acid (FFA) and triglyceride (TG). Resting heart rate and maximal oxygen consumption (VO2max) were measured. **Results**: In response to heparin injection, we observed a significant increase in FFA and significant decrease in TG ($P < 0.05$). VO2max was increased and resting heart rate decreased by heparin injection in experimental group ($P<0.05$). All variables remained without changes by lactose injection in control groups ($P>0.05$). **Conclusion**: Based on this data, it was pro concluded that increasing FFA availability by heparin infusion can improve fat oxidation and aerobic capacity during endurance exercise. Although, heparin injection to elevate free fatty acid concentration would not represent sound medical practice.

Keywords: Heparin, Fat oxidation, maximal oxygen consumption.

INTRODUCTION

Fats in forms triacylglycerol, intramuscular triglyceride, plasma triglyceride, very low density lipoproteins and fatty acids derived from the diet are considered the largest energy reserves during exercise, especially endurance exercises [1]. Depletion of glycogen reserves is identified as one of the factors limiting long-term exercise [2]. Carbohydrate and fat are the most prevalent substrates of energy during aerobic exercise [3]. At the beginning of exercise, the energy needed is mainly provided by the body carbohydrate sources, but with more activity, the contribution of carbohydrates in energy production gradually reduces and the contribution of fat increases [4]. Although it has been over 100 years since this was discovered, the mechanisms involved in controlling the uptake and oxidation of glucose and free fatty acids (FFA) during different activities, is not yet fully understood [4].
Endurance performance level is dependent upon the maximal oxygen consumption (VO2max) and carbohydrate or fat substrates [5]. VO2max is the body's ability to absorb more oxygen during exercise. The high level of this variable indicates a higher endurance capacity in an individual. Scientific studies have shown that the decrease glycogen reserves of muscle and liver is associated with reduced endurance performance and fatigue [5, 6]. Depletion of blood glucose or glycogen supplies are the main causes of fatigue during endurance activities [5, 6]. Unlike the limited reserves of carbohydrates, body fat sources are unlimited and are considered the inexhaustible source of fuel during exercise [1]. Scientific sources suggest that in a given endurance exercise, increased share of fat in energy production or increased FFA oxidation leads to decreased glucose consumption and accompanied with increase in endurance performance and delayed fatigue during such activities [2].

Several studies with the goal of increasing carbohydrates reserves and reducing its consumption during endurance activities, such as loading carbohydrates [7] and increase and consumption of plasma fat, in different way such as the use of medium-chain triglycerides [8], carnitine supplementation [9], intake of caffeine, arginine and other triglyceride decomposing supplements [4, 10, 11) and intravenous injection of fat and heparin solutions [12, 13] have been conducted or are running, but findings in this area are often contradictory and heterogeneous.

Heparin is a blood anticoagulant substance and stimulating lipoprotein lipase helps to enhance and accelerate the breakdown of triglycerides to FFA. Also its injection before exercise leads fat oxidation activity while starting the activity [14]. Some studies findings indicate that intravenous heparin infusion increases plasma FFA concentration [15]. This point must be considered that increased plasma FFA concentration due to injection of heparin or other supplements can not be regarded as an increase in fat oxidation in mitochondria. But some other studies directly indicate increased fat oxidation and reduction of glucose oxidation due to

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heparin injection before sporting activity [16]. Heparin injection before the exercise test in some other studies has also led to a 25% reduction of muscular glycogen consumption [17], increased FFA and decreased respiratory exchange ratio [18], decreased glucose consumption and maintaining the body's carbohydrate reserves during exercise, increasing the performance and runtime endurance and delayed fatigue. Despite these findings, some studies suggest that heparin consumption does not lead to a significant change in plasma FFA [19], blood glucose concentration [12, 13], carbohydrate and fat oxidation and endurance performance [20]. A review of the studies conducted in this area is suggestive of contradictory findings about the effect of intravenous injection of this substance on determinants of carbohydrates or fat oxidation and endurance performance and there is still no comprehensive consensus in this area. Some studies indicate the beneficial effects of heparin on the above mentioned variables and others suggest no effect on these variables during exercise. Hence, this study has been conducted with the aim to examine the effect of heparin intravenous injection on lipid metabolism determinants and maximal oxygen consumption which is considered a major factor in endurance performance.

MATERIALS AND METHODS

This single-blind clinical trial study was approved by the ethic committee of Islamic Azad University of Iran. This study determined the effects of heparin intravenous injection on free fatty acid, Triglyceride, maximal oxygen consumption and heart rate during cycling. On the other hand, the study objective was to determine whether heparin injection affects some physiological and metabolic marker during exercise in none-trained boy students. For this purpose, forty none-trained boy students (21±3 of age, 175±12 cm of height, 75±12 kg of weight) participated in study and divided into experimental (heparin injection) a control (placebo injection) groups by randomly. Informed consent was obtained from each subject after full explanation of the purpose, nature and risk of all procedures used. Subjects included individuals with no cardiovascular diseases, gastrointestinal diseases, kidney and liver disorders or diabetes. In addition, if any participants had been participating in regular exercise or diet program during the past 6 months, they were excluded study. All subjects were non-smokers. In addition, exclusion criteria included inability to exercise and supplementations that alter carbohydrate-fat metabolism.

In this study, all participants completed a graded bicycle-ergometry test (F90 Tuntury, Finland) according to Astrand protocol guideline (work load = 98 Watt) for 20 minutes [21] that conducted in two separate stages with an interval of 7 days. All individuals underwent the ergometry test after an overnight fast in two stages with an interval of 7 days. Firstly, all subjects of two experimental and control groups completed exercise test without injection of any substance. But in the second test, the subjects in the experimental group completed above mentioned exercise test 30 min after intravenous injection of heparin (10000 U). The subjects in the control group also completed exercise test within the same period; subsequent to intravenous injection of lactose as a placebo. Resting heart rate was monitored by a stethoscope and final heart rate of the exercise test was also recorded by Polar telemetry. Resting heart rate was recorded half time before exercise test. Maximal oxygen consumption (VO2max) in each test was calculated according to Astrand guideline. Subjects were told that avoid using the medicine or hormone preparations that affect the carbohydrate and lipid metabolism. Subjects were also asked to avoid doing any heavy physical activity for 48 hours before blood sampling. Immediately after each test, blood sampling was taken by a lab physician. These blood samplings used for measuring of lipid metabolism determinants such as FFA and triglyceride. The samples were centrifuged at 2000 rpm to separate serum. Triglyceride was measured by enzymatic method (Pars Azmoun, Tehran, Iran). Free fatty acids were measured using gas chromatography (Made in USA Varian Company; model 3800) (22).

Statistical analysis:

All values are represented as mean ± SD. Statistical analysis was performed with the SPSS software version 15.0. Comparisons of parameters between the two groups in first exercise test were made by independent sample T-test. Paired Student T-test was used to determine significance levels of changes in any of the variables in response to heparin or Lactose injection compared to first blood sampling in two groups. P-value less than 0.05 were considered statistically significant.

RESULTS

All subjects of experimental and control groups successfully performed the Astrand ergometry test. Table 1 shows the descriptive anthropometric, physiological and biochemical features of the study groups. There were no significant differences in all variables between in experimental and control group in pretest. Statistical results obtained from the paired T-test showed that VO2max increased significantly in experimental group ($p<0.05$), but this physiological variables did not change by lactose injection in control group ($P>0.05$) (Fig 1). Intravenous heparin injection led to a significant increase in serum FFA concentration ($P <0.05$), while lactose injection in the control group, did not bring about any significant changes in FFA concentration (Fig 2). Also the findings indicated a significant decrease in triglyceride by heparin injection in experimental group in the second exercise test compared to the first stage ($P <0.05$), while there was no significant change following lactose injection in control (Fig 3). On the other hand, resting heart rate which is among other factors that determine the cardiovascular fitness decreased (68±9 bpm versus 77±8 bpm) after heparin injection in the experimental group ($P <0.05$). All variables in control group remained without changes ($P>0.05$).

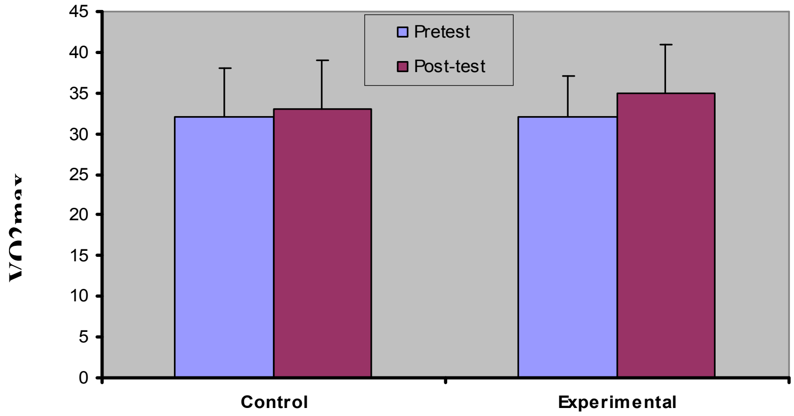

Fig 1. Mean and standard deviation of maximal oxygen consumption (VO2max) in response to Lactose and heparin injection after exercise cycling in control and experimental groups orderly

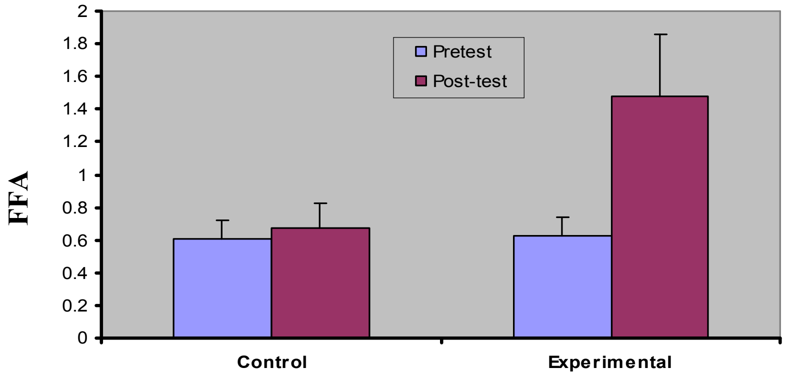

Fig 2. Mean and standard deviation of free fatty acid in response to Lactose and heparin injection after exercise cycling in control and experimental groups orderly

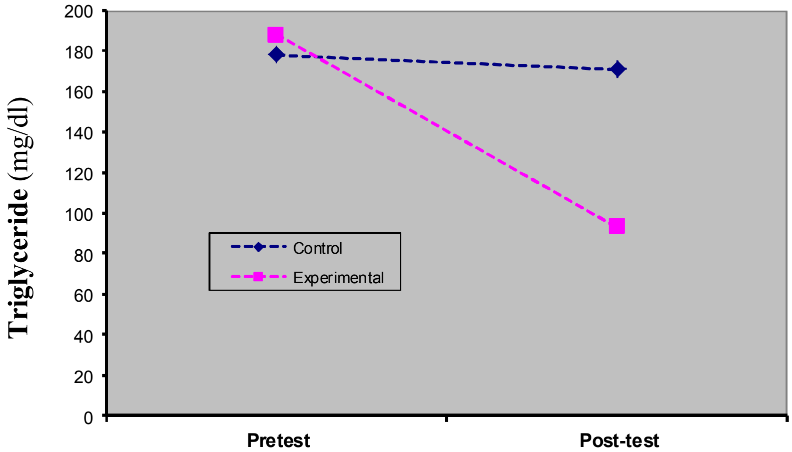

Fig 3. The changes pattern of triglyceride in response to Lactose and heparin injection after exercise cycling in control and experimental groups orderly

DISCUSSION

Although heparin intravenous injection may have certain advantages on the increase in plasma FFA levels without other hormonal changes, it is a violation of the International Olympic Committee doping regulation, this study was carried out only in order to further understand the mechanisms of biochemical reactions of fat - carbohydrate during exercise as this point has been mentioned in some other studies [23] and similar researches have been conducted with different methodologies. Since fat and carbohydrate are the main energy substrates during exercise, regulating the consumption of fat - carbohydrate in muscle tissue, has been the subject of many studies carried out by specialists of sports physiology and biochemistry. Some studies suggest that increased availability of lipid, leads to increased fat oxidation and decreased carbohydrate intake in skeletal muscles and the whole body during exercise [24]. The consequence of which is delay in the onset of fatigue and higher performance before the onset of fatigue. Increased availability of free fatty acid reduces the rate of glycolysis in heart and skeletal muscle [25]. Several factors such as increased capillary blood flow, the amount of intramuscular triglycerides [2, 26], the mitochondrial transmission of FFA, oxidative enzyme activity, hormonal adaptations susceptible to catecholamine and insulin and transformation or lipolysis of triglycerides to FFA and its transfer from blood to Sarcoplasm are effective in increase of FFA oxidation [2]. Although the increase in FFA plays an important role in the decrease of carbohydrates oxidation during long-term exercise, the findings on the effects of endurance performance are controversial. It must be mentioned that one of the main limitations of this study is failure to measure metabolic variables of carbohydrates metabolism such as glucose and lactate.

There is this hypothesis that the regulation of fat oxidation depends on two key stages, triglycerides lipolysis to FFA and its mitochondrial transport [3]. In this area, the Odland study (2000) showed that increased plasma FFA availability leads to increased mitochondrial transmission and fat oxidation [18]. Consumption of certain substances such as caffeine, arginine, coline, and ... as factors effective in decomposition of triglyceride to FFA during exercise, has repeatedly been studied and in some cases the findings suggest a positive role in the increased availability of FFA, decrease in plasma TG and increased endurance performance and aerobic capacity [10, 11, 27] but in some others no effect on plasma FFA concentration and endurance performance has been reported [28, 29]. The findings of another study showed that heparin injection leads to increased concentrations of FFA during rest, but its plasma concentration during exercise does not change compared with that before the injection [19]. Also the studies by Rantza (2007) and Everett (2006) showed that the increased availability of FFA caused by heparin injection has no effect on gluconeogenesis processes, plasma glucose, endurance performance and the other metabolic responses [12, 13, 20].

The findings of our study showed a significant decrease in resting heart rate, which represents one of the main signs of cardiovascular fitness in response to heparin injection or increased plasma free fatty acid. The most common variation by heparin injection is increased blood flow due its diluting characteristic as well as the dilation of blood vessels [30], which probably leads to an increase in stroke volume. Thus one likely reason for this significant decrease in resting heart rate can be attributed to the increased stroke volume caused by heparin injection. One of the properties of heparin is blood diluting property. It is therefore possible that the stroke volume increase due to blood dilution.

The results also showed that intravenous injection of heparin lead to increase in FFA concentration and decrease in Triglyceride during exercise. Increased FFA concentration can be the possible cause of reduction in triglycerides. With this interpretation that heparin injection brings about further breakdown of triglycerides to FFA, the consequence of which is the reduction in plasma triglyceride concentration. Increased availability of free fatty acid cause by intravenous heparin injection has been shown repeatedly [15, 16, 31]. It seems that this phenomenon is associated with increased fat oxidation, decreased glucose or carbohydrate intake [16, 17, 32] and increased fendurance duration [33]. Exercise with heparin pretreatment increased the total exercise duration from 6.5 to 9.5 minutes [34, 35] and also in patients with arthrosclerosis was associated with increased distance of running [36]. But some studies have reported no effect of increasing the FFA availability on plasma glucose concentrations [19, 20], triglycerides, heart rate, and oxygen consumption and endurance time [20, 37].

The findings of Ecano's study showed that the increase of plasma FFA leads to decrease in carbohydrate oxidation in early stages of the test but this decrease in carbohydrate oxidation is enough to increase or improve endurance during long-term exercise [37]. Some studies also suggest that despite increased FFA by heparin injection during exercise test, the other variables such as heart rate, respiratory exchange ratio, substrate consumption pattern and carbohydrate and fat oxidation does not change [20, 39, 38, 37].

It should not be forgotten that the increased availability of FFA or decreased plasma TG concentration alone is not decisive to increase fat oxidation, because fat oxidation is also dependent on the transfer of plasma triglycerides from fat tissue to plasma and is another key step that is mitochondrial FFA transport. Some findings suggest that increased plasma FFA concentration due to heparin injection does not correspond with the same increase in fat oxidation [40].

Conclusion

The findings of this study indicate the reduction of resting heart rate and increase in VO2max caused by heparin injection and these findings alongside the increase in plasma FFA to some extent support the positive effect of heparin infusion on endurance performance which is associated probably with increased fat oxidation. Understanding further the effective benefits of administration of heparin on athletic performance, requires additional laboratory studies measuring a wide range of metabolic variables involved in carbohydrate - fat metabolism for to identify new and comprehensive results.

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