Effects of 8 Weeks of Endurance Training on the Concentration of 6-IL, Insulin in Male Rats

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ABSTRACT

Skeletal muscle has the expression capacity of several cytokines, including IL-6, 8 and 15, together are called Myokine. In 2008, Handschin and Spiegelman have recognised the Myokines as the cytokines produced by the muscle cells that show the relationship between sport and inflammation Methodology: As long as the subjects of the different groups of this research were rats, which were in a controlled environment and at a pre and post planned test, under the effects of independent variable (8-week exercise program), so the research method is experimental. In the present research, after the initial agreement, fourteen 3-month-old male Wistar rats were obtained from the Pasteur Institute Centre of Amol. Variables measured in the exercise and control groups were compared and then the descriptive and inferential statistics were used to analyse the hypothesis test. Natural distribution of data measured by the Kolmogorov Simonov test, and the statistical analysis of the data performed using the software SPSS version 16 by the ANOVA test through repeated measurements based on the normal distribution. To compare variables between the two groups of the t test was used. The significant level for all calculations was considered as p <0.05. Results: According to table (1) and the independent t test significance level, the zero hypothesis is rejected. Therefore, eight weeks of endurance exercise has a significant impact on plasma levels of IL-6 in male rats (p≤0.005). There are significant differences in the concentrations of plasma insulin of rats with and without exercise. According to table and the amount of t and independent t test significance level, the zero hypothesis cannot be rejected. Therefore, eight weeks of endurance exercise has no significant impact on plasma levels of insulin in male rats (p≥0.005).

Key words:

Introduction

Skeletal muscle has the expression capacity of several cytokines, including IL-6, 8 and 15, together are called Myokine. In 2008, Handschin and Spiegelman have recognised the Myokines as the cytokines produced by the muscle cells that show the relationship between sport and inflammation [11,14]. Contractile activity is involved in the regulation of expression of high levels of cytokines in skeletal muscle. Myokines facilitates several cellular responses to exercise such as suppression of proteolysis, angiogenesis, and regulation of muscle glycogen [1,16]. By the way, the IL-6 has attracted much attention, because it is related to insulin during the exercise, i.e. during the increase of performance [15].

Therefore, this study intends to study the concentrations of IL-6, glucose over a period of endurance training on male rats. The time duration of endurance training activities may be longer, but conducted studies by Ghanbari rightly confirms this statement that the long-duration exercises with intensity of 60 to 80% Vo2max, especially for one or more weeks, would cause decreasing and depletion of energy reserves [5]. Woods et al also, concluded that the energy must always be balanced (between the delivery and expenditure of energy), so that the weight, the simplest indicator of the energy balance in a relatively long period of time remains balanced;
Interleukin 6 is a kind of cytokine that has the pre and anti-inflammatory effect. During exercise, the contracting skeletal muscle releases a certain amount of interleukin-6 into the bloodstream. The hypothesis is that IL-6, released from muscle, has metabolic roles (1, 2, and 5).

Due to the nature of its receptors, IL-6 has different effects on different cell types. The IL-6 has pro-inflammatory properties in fat cells and liver, leading to insulin resistance in both cells. Unlike the effects seen in the liver and fat cells, the positive effect of this type of cytokine on skeletal muscle by increasing glucose utilization has proved in short-term studies [13,22]. Release of insulin inhibitors appears counter when insulin is increased after exercise. Recent studies have shown that the IL-6 has anti-obesity effects and increases the insulin sensitivity, just like leptin that activates Kinase, which is an activator protein of 5AMPI (AMPK) in skeletal muscle and adipose tissue. Activation of AMPK has effects on the insulin signalling pathway and will increase the glucose utilization [4,22]. As mentioned, the place of generation and effectiveness area of cytokines is beyond the range of immune system. Exercise is an interesting example to demonstrate how cells with no immune source, can produce and discharge particular cytokines [11].

The expression of intramuscular interleukin-6 and release of muscle proteins will increase when the intramuscular glycogen is in critical situation. It seems that interleukin-6 is somewhat related to muscle glycogen content. Release of IL-6 from the contracting muscles, following with accumulation in the general circulation is closely associated with the duration of exercise [8,21]. During long-term exercise, the glycogen levels of the contracting skeletal muscle are reduced, so it is hypothesized that long-term exercise, and in response to the energy crisis, particularly reduction of glycogen stores of Myofibers of contracting muscles, the IL-6 will release from the muscles. By reducing the muscle glycogen, the dependency of contracting muscles to the blood glucose, as an energy source, will increase and so the release of IL-6 from the muscles might be a signal to the liver to increase glucose production to prevent the reduction of blood glucose due to the exercise [4,10, and 11].

According to some studies, the glucose infusion during exercise reduce the increase rate of serum interleukin-6v, yet, although the complement of carbohydrates during the exercise inhibits the increase of serum IL-6, but has no effect on expression of increasing IL-6 in contracting muscles [6,9,12].

IL-6 release from contracting muscle during exercise provides a signal to produce liver glucose, regulates the muscle substrates consumption, and enhance the glycogen sources and fat oxidation.

Interleukin-6
The word Cytokines is driven from the Greek words cyt- (meaning Cell) + -kine (as movement). Cytokines are proteins or peptides as the molecules that produced and released by the immune system and are mediated for immune responses. Rather, cytokines are excretion molecules with special effects for the cell that has been be secreted from as well as the other cell. Although initially cytokines have identified as molecules that are released by the immune system, but today their place of generation and the scope of effects is beyond the immune system. Cytokines release form cells called T. the auxiliary T cells are the maximum number of T cells, and are usually more than \( \frac{3}{4} \) of the whole. The cells assists the immune system and do it through different ways. In fact, virtually all are the major regulators of the immune system [11], is a cytokine which its main source in the body is activated macrophages, fibroblasts, and endothelial cells, however, other cell sources for IL-6, such as T And B cells, Neutrophils, Eosinophils, Osteoblasts, Granulocytes and Miocytes are identified. Therefore it seems that the release of IL-6 from contracting muscles and, consequently, accumulates in the general circulation is closely associated with the exercise duration. During long-term exercise, the contracting skeletal muscle glycogen levels is reduced, so it is assumed that IL-6 releases from the skeletal muscles during the long-term exercise and in response to the energy crisis, particularly in the reduction of contracting myofibrils muscle glycogen stores. By reducing the glycogen in muscle, the dependency of contracting muscle on glucose as an energy source increases, and so, the release of IL-6 from the muscles might be a signal to the liver to increase glucose production to prevent the reduction of blood glucose due to the exercise. Stimulation of cortisol secretion by IL-6 may be due to the effect of IL-6 on hypothalamus and stimulates the release of ACTH from the anterior pituitary gland, or through the direct effect of IL-6 on release of cortisol from the adrenal gland. Relatively small increase in plasma levels of IL-6, stimulates the IL-10 anti-inflammatory cytokine and IL-1RA with CRP. Exercise causes fixed changes in leukocyte sub categories; so the number of neutrophils increased during and after exercise. Injection of yhIL-6 also will cause similar effects that are likely by mediated cortisol. Lymphocytes initially increase during exercise and after exercise decrease, while these changes may be because of catecholamines. However, long-term reduction in lymphocytes may be because of increase in plasma IL-6 due to exercise. Based on these findings, IL-6 released from the muscle play a role either in regulation of metabolism or in regulating the immune system. It has been proven that in addition to the contracting muscles, other tissues participate to increase concentration of systemic IL-6 due to the exercise. [11,1,13].

otherwise, the balance will disturb and over or under weight will occur [12].
Insulin:

First time in 1922, Banting and Best, achieved insulin from Islets of Langerhans in pancreatic. Insulin is a small protein. The molecular weight of human insulin is 5808. It has two amino acid chain that disulfide bonds have connected them together. When these two chains separate, insulin molecule loses its activity. Insulin in Beta cells is produced in the same manner of the protein’s construction, such that, first the ribosomes bound to the endoplasmic reticulum translate the RNA of Insulin to produce the insulin prohormone, that its molecular weight is about 11,500. Its cut-off in the endoplasmic reticulum leads to the production of proinsulin, which has a molecular weight of about 9000. Much of proinsulin after cutting converts to insulin in the Golgi body [7]. Then, the insulin is packaged in secretory granules. However, about 1/6 of final secreted production is in form of proinsulin. Proinsulin does not work as an insulin. Almost all insulin secreted into the blood will be transported without binding to proteins; the plasma half-life is only about 6 minutes and thus will be cleaned of blood completely within 10 to 15 minutes, unless only part of insulin that binds to receptors on the target cell; the rest will be broken by insulinase enzymes, that much of it is in the liver, some in kidneys and muscles, and slightly less in other tissues. The rapid removal of insulin from plasma is important because sometimes a quick stop of the effects of insulin is as important as insulin control measurements. Insulin facilitates the consumption of carbohydrates to produce energy and at the same time reduce fat intake. In contrast, when insulin is not available fats become the main source to provide energy (except the brain that uses glucose). The main reason for the shift of energy source is glucose density in the blood. When the blood glucose density is low, insulin secretion decreases and fat is consumed for energy in the body tissue. When the glucose levels are high insulin secretion increases and glucose is consumed for energy; therefore the important role of insulin in the body is providing cellular energy source at any moment. Other than increased blood glucose, arginine and lysine amino acids and gastrointestinal hormones such as gastrin, secretin, cholecystokinin, gastric inhibitory peptide, and growth hormone, cortisol and glucagon have an important role in insulin secretion.

When the blood glucose levels fall between meals, some event occurs so that the liver to release glucose into the bloodstream [7,22].
- Fallin blood glucose levels reduces insulin secretion from the pancreas.
- Decreased insulin reverses all the effects that are associated with the storage of glycogen; production of glycogen in liver stops and glucose does not transfer from the blood to the liver cells.

- Decreased insulin activates the phosphorylase enzyme to break the glycogen to glucose phosphate.
- The phosphatase glucose enzyme which was inhibited by insulin had been activated due to lower insulin and separates the phosphate radicals from the glucose, so the free glucose can be released into the blood [2,7]. This will end to the cell consumption of glucose and thus the blood glucose level will reduce. Moreover, the action of glucose will prevent the release of glucose from the liver and free fatty acid, from the fat tissue [2].

Rovasi et al (1384) have studied the effects of endurance exercise on proinflammatory cytokines and insulin resistance in obese men, and reported that endurance exercise reduces the TNF-a, and IL6 baseline as well as insulin resistance indicator [22].

Pedersen et al (2011) have examined the presence of liver renin enzyme (C × CL-1) during exercise which depends on the amount of IL-6 were attached to the muscle. Following a specific exercise, the protein C × CL-1 increased 2.4 times in the serum and MRNA C × CL-1 increased 6.5 times in muscle and 41 times in the liver. In contrast, by stopping the practice the MRNA C × CL-1 of the liver completely was stopped in IL-6 of rats, which caused an increase in the C × CL-1 of serum for 5 times and 24 times in the liver compared with the control group. The results obtained from this study were that the hunger, significantly, increases C × CL-1 of serum and presence of C × CL-1 in the liver and the muscle; and also it has proven that the liver is the main source of C × CL-1 of serum during exercise levels in rats [18].

Holmes et al (2008) have presented a research to show the chronic increase in IL-6 by external injection of IL-6. They assumed that the continuous increase in IL-6 is a preventing and inhibiting factor for glucose tolerance and insulin sensitivity; while the severe intermittent will improve it. The Wistar male rats treated for 14 times by human IL-6 (2.4 mg per day) or by saline solution with low osmotic activity or by twice a day periodic injection of IL-6. The tests of glucose and insulin tolerance implemented on the rats, after 14 times of treatment and 24 hours after the injection of Insulin (150 mg) or saline solution. Approximately 10 minutes after insulin injection, pieces were taken from the muscle and liver. The IL-6 treatment increased the basic insulin sensitivity, as insulin resistance already had been measured by the homeostasis measurement, and elevated the presence of glucose during the glucose tolerance test. The IL-6 increased free fatty acids, but did not increase triglyceride accumulation in skeletal muscle or liver, whereas either the Alpha protein or VCP skeletal muscle increased. This fact indicates that IL-6 can increase fat oxidation by mitochondria. The result is that, regardless of the delivery mode, IL-6 treatment during 2 weeks enhances glucose tolerance[9].

Anderson et al (2008) investigated the possible role of IL-6 in vascular insulin resistance and
concluded that the endothelium plays an important role in regulating the vascular mode through the release of nitrous oxide to the smooth muscle of vessels, which plays a role in energy supply through the regulation of distribution of blood flow in different tissues. TNF-a increases the IL-6 production; there is a relationship between the bad function of Andoselia and these cytokines. The result is that according to the vascular insulin resistance, the available data indicates the direct pathology role of TNF-a in bad function of Andoselia [3].

Pedersen et al (2001) studied the effect of exercise on cytokines, especially muscle IL-6, and found that exercise stimulate the increased level of a number of cytokines. Therefore in the plasma increased level (TNF-a), Beta IL-1, IL-1ra, TNFr, IL-10, IL-8 and deflationary macro-cellular proteins (MTP-1) can be found after intense exercise. Also the concentration of IL-6 after a marathon race has increased 100 times. The result of these findings were that, IL-6 produces in response to exercise more than any other cytokines. Also IL-6 is known for stimulation of output glucose and stimulation and increase of lipolysis, which means that IL-6 can represent an important link between contracted skeletal muscle and metabolic changes related to exercise [17].

Method:

As long as the subjects of the different groups of this research were rats, which were in a controlled environment and at a pre and post planned test, under the effects of independent variable (8-week exercise program), so the research method is experimental. In the present research, after the initial agreement, fourteen 3-month-old male Wistar rats were obtained from the Pasteur Institute Centre of Amol.

After completion of training and 48 hours after cessation of exercise, and after 4 hours of fasting from food, the rats were anesthetized by intraperitoneal injection of a mixture of ketamine and xylazine. The liver tissue was cut off immediately and placed in liquid nitrogen and then the tissue were homogenized with 17 mm phosphate buffer and with a speed of 8000 rpm. The blood samples centrifuged immediately for 10 min at 1500 rpm. The plasma transferred in special micro tubes (3 samples of each) and became frozen in liquid nitrogen, and was maintained for subsequent measurement in the freezing of the temperature of -80°C to avoid the evening effects, sampling began from 8 am and was completed 11:30 am. Variables measured in the exercise and control groups were compared and then the descriptive and inferential statistics were used to analyse the hypothesis test. Natural distribution of data measured by the Kolmogorov Simonov test, and the statistical analysis of the data performed using the software SPSS version 16 by the ANOVA test through repeated measurements based on the normal distribution. To compare variables between the two groups of the t test was used. The significant level for all calculations was considered as p <0.05.

Results:

There are significant differences in the concentrations of plasma IL-6 of rats with and without exercise:

The zero hypothesis (H0):

There is no significant differences in the concentrations of plasma Insulin of rats with and without exercise.

According to table (1) and the independent t test significance level, the zero hypothesis is rejected. Therefore, eight weeks of endurance exercise has a significant impact on plasma levels of IL-6 in male rats (p≤0.005).

There are significant differences in the concentrations of plasma insulin of rats with and without exercise.

The zero hypothesis (H0):

There is no significant differences in the concentrations of plasma insulin of rats with and without exercise.

<p>| Table 1: The independent t test to compare the Plasma IL-6 of rats with and without exercise |
|-----------------|----------|---------|--------|--------------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>variable group</th>
<th>mean</th>
<th>Standard deviation</th>
<th>Amount of t</th>
<th>Degrees of freedom</th>
<th>Significant level</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>control group</td>
<td>82.29</td>
<td>23.39</td>
<td>-4.658</td>
<td>12</td>
<td>0.001</td>
<td>Reject the zero hypothesis</td>
</tr>
<tr>
<td>practice group</td>
<td>138.29</td>
<td>21.55</td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

* In the level of p ≤ 0.05 significant

<p>| Table 2: The independent t test to compare the Plasma insulin of rats with and without exercise |
|-----------------|----------|---------|--------|--------------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>variable group</th>
<th>mean</th>
<th>Standard deviation</th>
<th>Amount of t</th>
<th>Degrees of freedom</th>
<th>Significant level</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.17</td>
<td>0.098</td>
<td>-0.699</td>
<td>12</td>
<td>0.498</td>
<td>Does not reject the zero hypothesis</td>
</tr>
<tr>
<td>practice group</td>
<td>0.14</td>
<td>0.058</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Accord ing t o table (2) and the amount of t and independent t test significance level, the zero hypothesis cannot be rejected. Therefore, eight weeks of endurance exercise has no significant impact on plasma levels of insulin in male rats ($p \geq 0.005$).

Discussion:

The first result obtained from this study is increase of plasma levels of IL-6 in trained male Wistar rats compared with rats with no exercise, which were statistically significant ($p=0.001$).

Recently observed that IL-6 plays an important role in regulation of glucose supply and increase of genes associated with the gluconeogenesis process, and when the active skeletal muscles need energy, the increase in IL-6 through the active muscles could possibly increase the output of liver glucose. Olarense n et al (2002) using repeated series of exercise on the plasma levels of interleukin-6 and IL-1 receptor antagonists, concluded that interleukin-1 and interleukin-6 responses were increased which can be attributed to the depletion of muscle glycogen [21]. Also Steensberget al (2000) studied effect of interleukin-6 in humans contracting muscles, and have found that the net IL-6 in the muscle during two hours after the practice had increased seventeen times compared to the concentration of arterial IL-6. This increase was related to the type and duration of exercise [5]. Niemann and colleagues (2006) studied the amount of cytokine mRNA of the white blood cells after reducing the exercise in 12 athletes by injection of a carbohydrate drink (placebo), which have faced with the reduction of muscle IL-6 during exercise (by placebo injection), but increased IL-8, IL-10 and IL-1ra. This research is not in agreement with the present study, and it is because of the food type and supplements that were injected to the cases [22]. Chan and colleagues (2004) have studied the amount of cytokine stimulation levels in human skeletal muscle during intensive contraction and concluded that the concentrations of interleukin 6 and 8 is increased muscle during exercise. The frequency of IL-6 and 8 through the glycogen store in in concentrated muscle is affected [13]. Fischer et al (2004) defined ten-week endurance stretch of the knee to study the presence of IL-6 MRNA in skeletal muscle of seven healthy young men in response to three hours of active exercise of stretching knee, using the same activity pressure. Results obtained from this study was that the presence of IL-6 MRNA in human contracted skeletal muscle was significantly reduced by ten weeks of exercise, which is because of the type of activity. In the research by Ro vasi et al (2004), the muscle IL-6 decreased, which is not consistent with the present study [4].

Also, the results of this study about changes in plasma insulin levels of male Wistar rats did not report any significant changes in them. In this regard, Peterson et al (2005) were examined anti-inflammatory effects of regular exercise on IL-6 and TNF-a. The results obtained from this study was that regular exercise reduces TNF-a suppression and elimination, and is a protection against TNF-a decreased insulin resistance, which is because supplements are consumed before exercise. Lork et al (2009) in a study of 37 diabetic patients following cardiac surgery, were measured an index of glycemic insulin by assessing the need for insulin to maintain evglycaemia and repeated measurements. Insulin resistance increased during the observing period and 22 hours after initiation of treatment. In conclusion, serum cortisol level, is the best predictor of anti-insulin resistance, after IL-6, leptin and adiponectin. This relationship may be due to the fact that TNF-a and resistance have little relevance for predicting the stress associated with insulin resistance [13].

This research is not in agreement with present study, because the increase in plasma insulin was significant. Clover et al (2003) in a 5-day constant use, showed that before intravenous insulin action, the IL-6 will be the first insulin receptor in the liver of rats; and 6 times increase in IL-6 levels by constant injection, was similar with the obtained level of obesity. The result was that the insulin resistance indicates the reduced insulin sensitivity. Unlike the shift of the hepatic insulin receptor, a five-day reception of interleukin 6 fails to overcome the shift of skeletal muscle insulin receptor. This is because of the type of food for subjects. Also Carey and colleagues (2006) showed that IL-6 intake can affect the body's glucose levels. The results obtained from this study was that acute IL-6 treatment, increases the amount of stimulated glucose of insulin in relatives, while the effects of IL-6 on glucose and fatty acid metabolism by AMPK may be imposed. Which is because of the amount of IL-6 consumption [4].

Conclusion:

The results show that an eight-week endurance training can be considered as an effective factor for the immune and humoral system. Endurance exercise seems to be effective on plasma levels of IL-6 in male rats. The results of this study can be considered as a potential candidate to change the size of IL-6 in plasma of rats, and despite minor differences in the effects of endurance exercises on insulin of male Wistar rats, these results should be carefully considered and analyzed.

References

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