IL-6 RESPONSES TO GLYCAEMIC INDEX DURING RECOVERY FROM EXERCISE
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Abstract. **Purpose:** This study examined the effect of meal with different glycaemic index (GI) on plasma IL-6 concentration and glucose metabolism after maximal lengthening contractions of the knee extensors. Using a cross-over design. **Material:** 10 healthy males completed 5 sets of 10 lengthening (eccentric) contractions at 120% 1 repetition-maximum. Subjects were randomized to consume the GI beverage (high-GI, low-GI (15% weight per volume; 3 g/kg BM) or placebo in three times within 10 min following exercise, and again at 50 and 110 min during recovery time. Blood samples were collected before exercise and after 0.60, 180 min and 24 h of recovery. **Result:** Concentration of plasma IL-6 in HGI group was less than LGI and Pla groups. IL-6 tended to significantly increase after exercise in recovery time in 3 groups (all P < 0.05), except for 24 hours (P = 1.00), furthermore there was significant difference for IL-6 between placebo and high glycemic groups in 3hours after exercise (P=.016). Concentration of serum CK in HGI group was less than LGI and Pla groups, CK was significantly elevated at all times points during recovery in 3 groups (all P < 0.05), except for 1 hour after exercise in HGI group (P = 0.31), but there was no significant difference for CK between groups. **Conclusion:** In summary, consuming HGI carbohydrate during recovery from exercise attenuate plasma IL-6 concentration.

Key words: Exercise, Glycaemic, index, carbohydrate, plasma.

Introduction
Eccentric skeletal muscle contractions induce inflammation and damage within muscle directly (Beaton et al., 2002), whereas indirect markers include loss of muscular strength and increased release of myocellular proteins (creatine kinase) into the circulation (Hirose et al., 2004; Paulsen et al., 2005; Peake et al., 2006). In response to exercise-induced muscle damage, neutrophils and macrophages are mobilized into the circulation (Paulsen et al., 2005; Pea et al., 2006), and subsequently infiltrate damaged muscle tissue (Beaton et al., 2002; Raastad et al., 2003). The systemic concentrations of cytokines (IL-6) also increase following muscle injury (Smith et al., 2000; Nieman et al., 2004; Paulsen et al., 2005; Peake et al., 2006). Eccentric exercise is associated with an increase in IL-6 (Steenberg et al., 2002) and can be used as a model to induce muscle damage and inflammation.

Carbohydrate (CHO) metabolism is a key factor that could influence on immune responses during exercise (Nieman et al., 2006). During endurance running and cycling, carbohydrate ingestion reduces circulating post-exercise leukocyte counts, and the plasma concentrations of IL-6 (Nieman et al., 1998, 2003, 2005). Within skeletal muscle, CHO suppresses IL-6 and IL-8 mRNA expression after endurance running (Nieman et al., 2003), but not in response to endurance cycling (Febbraio et al., 2003; Nieman et al., 2005). Consumption of CHO before and during 2 h intense resistance training reduces circulating neutrophil counts (Nieman et al., 2005). The immune response to CHO ingestion during the early recovery period following intense resistance exercise is currently unknown. These findings indicate that glucose exerts anti-inflammatory effects during exercise, when metabolic demands are high. Glucose may influence inflammatory responses within blood and skeletal muscle differently when CHO is consumed in the recovery period following muscle injury, when metabolic stress is lower and inflammation is higher (Louis et al., 2007). Evidence exists that large doses of glucose can induce inflammation and oxidative stress at rest in normal healthy individuals (Mohanty et al., 2000; Dhindsa et al., 2004; Aljada et al., 2006; Dasu et al., 2007; Dickinson et al., 2008).

Glycemic index (GI) describes the difference by ranking CHO according to their effect on blood glucose levels(Jenkins et al., 1981). Different type of CHO and exercise may have various effects on immune function (Kirwan et al., 2001). Resistance training with high-force eccentric contraction causes more micro-tears in muscle and more inflammatory responses than concentric contraction (Miles et al., 2010), also one of the most important aspects of recovery from resistance exercise, that can be influenced by nutrition is the synthesis of muscle glycogen to replace stores lost during exercise and thus can be used as a model to study the influence of diet on the inflammation process. The inflammation might be increase with a high amount of CHO diet following a high-force eccentric contraction (Miles et al., 2010). On the other hand a high GI diet following exercise increased inflammatory responses during recovery in resistance exercise, could guide the athletes for choosing beneficial nutrition to reduce their damage. Few studies, if
any, have investigated the influence of a GI beverage on IL-6 response during recovery from resistance exercise. We also examined the effect of a post-exercise beverage with different GI on IL-6 responses to intense resistance exercise. The aim of this study was to determine whether there are differences in plasma IL-6 concentration between low and high GI during recovery from resistance exercise.

Materials and Methods
Subjects
Ten weight lifter males (age 22±2 years; body mass 83±10 kg; high 177±5 cm; fat percentage (%) 12±2; 1RM 235±42/kg and training experience 4±1years) were volunteers to participate in the study, volunteered to participate in this study which was approved by the Ethical Committee of Qom University of medical science. Written informed consent was obtained from all subjects. They were also required to complete a general health questionnaire and were excluded if any medication had been taken during the 6 weeks prior to the study and if symptoms of upper respiratory infection had been experienced in the 4 weeks prior to the study. Moreover, at the time of the study, all subjects were involved in normal training (2–3 times per week). Subjects were asked to refrain from alcohol consumption 24 hours prior to sample collection.

Preliminary testing
1RM assessment
At least 1 week prior to main trial all subjects reported to the laboratory to assess leg strength. Maximal dynamic strength 1RM was determined using a 60° incline heavy duty leg press. Following a 5-min warm up on a stationary bicycle, each subject was given 6 lifting attempts in order to achieve their 1RM. A valid repetition involved lowering the weight to the point of 75° of knee flexion (the load-lowering point) as measured by a Jarma goniometer (Therapeutic Equipment, Clifton, NJ), and then extending to full leg extension. Subjects were instructed to rest for 2–5 min between repetitions to ensure a true maximal lift was achieved. After each successful lift, the load was increased by 2.5–5 kg until failure to complete one repetition. Subjects were given a maximum of 2 attempts to lift the weight; the greatest amount lifted successfully was recorded.

Exercise familiarization
Subjects were familiarized with the resistance exercise protocol, in which they were required to resist the downward movement of the weights. This movement involved lengthening (eccentric) contractions of the knee extensor muscles. A hand winch (5:1 ratio; model number: F10217; line pull capacity: 700 kg; Jarrett Synergy, SA, Australia) was fitted to the frame of the leg press, so that when the weight was lowered to the load-lowering point, the winch was used to lift the weight and reset it for the next repetition. In this fashion, the subjects only completed lengthening muscle contractions, with no shortening (concentric) contractions. Before the main trials, participant kept a 3-day diary of their dietary intake before the main trial and energy intake and dietary composition were subsequently analyzed (The Food Processor 10.0, Esha). They were required to repeat the same diet before each main trial to minimize the variation in muscle and liver glycogen concentrations.

Experimental procedure
This study is a counterbalanced cross-over design and the order of the three trials will be randomized, separated by at least14 days. Subjects were randomized to consume the GI beverage: High-GI (GI = 83) glucose drink 15% weight per volume; 3 g/kg body mass GI = 83), Low-GI (GI = 36) fructose drink 15% weight per volume; 3 g/kg body mass GI = 36) or placebo(an equal volume of flavor- and color-matched artificially sweetened placebo)( Ross et al., 2010), in three time within 10 min following exercise, and again at 50 and 110 min during recovery time.

On the day of the main trial, between four and seven days after the first visit the subjects were brought to the laboratory at about 8:00 am after an overnight fast of 12 h. After collection of the baseline blood samples the subjects were repeated the standardized warm-up on a stationary bicycle. The resistance exercise protocol consist of 5 sets of 10 lengthening contractions (5 sets, 10 repetition in each set) at a workload of 120% 1RM legs press. Each set was separated by a 2-min rest interval. Subjects were instructed to lower the load in a controlled fashion to a 75° angle at the knee joint over 5 s (digital stopwatch, Cal. SO56, Seiko, Australia). During recovery from resistance exercise, subjects were consumed either the GI beverage: High-GI (GI = 83 ) glucose drink 15% weight per volume; 3 g/kg body mass GI = 83), Low-GI (GI = 36) fructose drink 15% weight per volume; 3 g/kg body mass GI = 36) or placebo(an equal volume of flavor- and color-matched artificially sweetened placebo)( Ross et al., 2010), in three time within 10 min following exercise, and again at 50 and 110 min . All of the trials were performed under similar conditions of barometric pressure, temperature, and relative humidity. No extra food was allowed until after the final blood sample was been taken.

Blood analyses
10 ml venous blood will be drawn from an antecubital vein in the forearm at each time point: 1- Fast blood sample (F.B.S) , 2- Immediately post-exercise, 3- 1 hour 4- 3 hours after exercise 5-24 hour after exercise during recovery period, into two different evacuated collection tubes (Vacutainer; Becton Dickinson, Mountain View, CA). The first (5 ml) venous blood sample was drawn into a vacuum tube with clot activator and serum separator for collection of serum to analyze glucose by BIOSEN C (EKF Diagnostic GmbH, West Germany) and CK by SYNCHRON® System(s) (Beckman Coulter, Inc.USA). The second (5 ml) collection of serum to analyze glucose by BIOSEN C (EKF Diagnostic GmbH, West Germany) and CK by SYNCHRON® System(s) (Beckman Coulter, Inc.USA). The second (5 ml) venous blood sample was taken in to K3 EDTA vacutainers .5ml blood sample was spun at 1500 g for 10 min at 4°C to obtain plasma which was immediately stored at -70 °C before being analyzed for IL-6. IL-6 was determined with the use of quantitative sandwich type enzyme-linked immunosorbant assay (ELISA) kits from R&D systems (Minneapolis, MN). All standards and solutions
were prepared, and procedures were followed according to the kit specifications. Samples were diluted when necessary to ensure that the measurement fell within the range of the standard curve.

Data analysis

All collected data will be presented as mean and standard deviation (Mean ± SD). Repeated measures 5 × 3 (time × groups) ANOVAs was used to assess metabolic and immune differences between groups. Any significant F ratios shown were assessed using Bonferroni correction test. Assumptions of homogeneity and sphericity in the data were checked. Statistical significance was accepted at p < 0.05. The data was analyzed by using the statistical package SPSS, PC program, version 19.0 (SPSS Inc., USA).

Results:

10 Subjects completed 3 sessions of resistance exercise (Table 1). The protocol consist of 5 sets of 10 lengthening contractions (5 sets, 10 repetition in each set) at a workload of 120% 1RM) legs press.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>M± SD</th>
</tr>
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<tbody>
<tr>
<td>Age (yrs)</td>
<td>22±2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177±6</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>83±10</td>
</tr>
<tr>
<td>Fat percentage (%)</td>
<td>12±2</td>
</tr>
<tr>
<td>1RM (kg)</td>
<td>235±42</td>
</tr>
<tr>
<td>Training experience (yrs)</td>
<td>4±1</td>
</tr>
</tbody>
</table>

Markers of inflammation

Plasma IL-6 concentration and CK were measured as indicators of inflammation as a result of muscle damage. For IL-6 at the baseline, there was no significant difference between trials. Concentration of plasma IL-6 in HGI group was less than LGI and Pla groups. IL-6 tended to significantly increase after exercise in recovery time in 3 groups (all P < 0.05), except for 24 hours (P = 1.00), furthermore there was significant difference for IL-6 between placebo and high glycemic groups in 3hours after exercise (P=.016) (Fig.1). At the baseline, there was no significant difference for CK between trials. Concentration of serum CK in HGI group was less than LGI and Pla groups, CK was significantly elevated above baseline values at all time points during recovery in 3 groups (all P < 0.05), except for 1 hour after exercise in HGI group (P = 0.31), but there was no significant difference for CK between 3 groups (Fig.2).

Fig. 1. Mean concentration of plasma IL-6 for Pla, LGI and HGI diet groups at the various time points. Significant differences between Pla and HGI (treatment effect) are indicated with (**) where P < 0.05 Significant differences from baseline (time effect) are denoted by (*) where P < 0.05. Solid black bar represents resistance exercise bout; Pla = placebo, LGI = Low glycemic index, HGI = High glycemic index. Values are mean ± SE.
**Blood glucose:**

The serum concentration of glucose was measured to identify differences in the diets between conditions. At the baseline, there was no significant difference for glucose between trials. Glucose increased significantly following carbohydrate ingestion. Concentration of glucose in HGI group was more than LGI and Pla groups, glucose was elevated above baseline values at 1h after exercise in HGI and LGI groups (all P < 0.05). There was significant difference for glucose between placebo with low glycemic groups (P=0.00) and placebo with high glycemic (P = 0.00), also high glycemic with low glycemic groups (P = 0.00) in 1h after exercise. Furthermore there was significant difference for glucose between placebo with high glycemic (P=0.00) and also high glycemic with low glycemic group (P = 0.00) in 3h after exercise (Fig.3).
Discussion:

The aim of this study was to determine whether there are differences in plasma IL-6 concentration between low and high GI beverage during recovery from maximal lengthening contractions of the knee extensors. The key finding of this study was that plasma IL-6 concentration decrease significantly in HGI group to compare with LGI and Pla groups at least during the early post-exercise recovery period.

We selected an exercise model that would induce moderate muscle damage with relatively little metabolic stress in skeletal muscle. Generally, eccentric exercise elicits an inflammatory response for repair and adaptation, leading to increases in IL-1 and IL-6, while anti-inflammatory cytokines appear to be down regulated and inhibited (Peake et al., 2005). In current study IL-6 significantly increased after exercise in three groups which was expected, as exercising skeletal muscle is a powerful stimulator of IL-6 (Petersen et al., 2005). No previous research has investigated cytokine responses during the early phase of recovery from eccentric contractions in different glycemic trials. The glycemic index is a scale that indicates how rapidly different foods influence blood glucose levels. The purpose of elevating blood glucose to the point of hyperglycemia in the high carbohydrate condition was because previous studies indicate that hyperglycemia can induce and or augment inflammation (Esposito et al., 2002; Gonzales et al., 2006). The present data demonstrate that concentration of IL-6 in HGI group was significantly less than LGI and Pla groups. Inflammation following muscle injury is important for tissue regeneration, but excess inflammation may delay tissue regeneration.

Muscle injury combined with hyperglycemia attenuated plasma IL-6 concentrations. (Stevenson et al., 2005). Depner and colleagues (2009) lend tentative support for these findings. They reported that consuming a high-carbohydrate meal after 60 maximal lengthening contractions of the elbow flexors tended to raise the plasma concentrations of IL-6 compared with consuming a high-protein/fat meal.

Creatine kinase is indicative of loss of muscle integrity, and researchers have suggested that muscle integrity is most compromised in high force eccentric contractions (Eston et al., 1995). We also measured the serum concentrations of CK in recovery time after exercise; we observed that the serum concentrations of CK increased after exercise during recovery period. These findings complement data from other studies indicating that plasma/serum cytokine concentrations generally increase beyond 3 h after resistance exercise (Smith et al., 2000), and lengthening contractions.
of the leg muscles (MacIntyre et al., 2001; Paulsen et al., 2005) and arm muscles (Hirose et al., 2004; Miles et al., 2007). According to our finding CK in HGI group was less than LGI and Pla groups, but not significantly. This increase in CK peaked at 24 hours post-exercise for the HGI group, which is consistent with previous research on downhill running (Eston et al., 1995).

CK can be elevated for several days in individuals (Miles et al. 2010). The exercise protocol in our study included only 50 single-leg contractions, whereas other studies have used upper and lower body exercises (Smith et al., 2000), and 200 single-leg contractions (MacIntyre et al., 2001; Paulsen et al., 2005). The smaller muscle mass and lower number of contractions used during exercise may also account for the lack of any substantial systemic inflammatory response after exercise.

Conclusions
We can conclude that there is consuming of HGI carbohydrate during recovery from exercise attenuate plasma IL-6 concentration. Future research could investigate the effects of consuming carbohydrate with different glycemic index during recovery from other forms of exercise such as prolonged and interval, which induces greater muscle injury, that require higher intake of carbohydrate to restore muscle glycogen content. Interferences aimed at diminishing excess inflammation may promote faster muscle repair and recovery of muscle function.

Conflict interests
The authors declare they have no conflict interests.

References


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