The Difference Effect of Low and High Glycemic Load Diet on Immune Responses of Adolescent Soccer Athletes

by Liani Setyarsih

The Difference Effect of Low and High Glycemic Load Diet on Immune Responses of Adolescent Soccer Athletes

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Abstract. High-intensity physical exercise causes physical stress that will suppress the immune system in athlete's body. Decreased immune system function will increase risk of infection and reduce athlete performance. Regulation diets of glycemic index (GI) and glycemic load (GL) are known to help control blood glucose during exercise so the immune system can be maintained. The purpose of this study was to determine differences effects of low and high glycemic load diets on immune responses in adolescent football athletes. This study was a quasi experimental pre-post-test group design, conducted on 22 adolescent football athletes aged 15-17 years old. The subjects were divided into two groups, low GL diet group was given carbohydrate-source foods with GL of 9.15, high GL diet group was given foods with GL of 27.29. Diet was given once in the morning and 2 hours later subjects doing RAST (Running-based Anaerobic Sprint Test) to trigger physical stress. Immune response was measure using total leukocytes and leukocytes differential count. There were no significant differences in blood glucose levels, leukocyte counts, and leukocytes differential count between low GL and high GL groups (p>0.05). Low GL diet causes an increase in blood glucose and total leukocytes smaller than high GL diet.

Keywords : glycemic load diet, immune responses, leukocytes, leukocyte differential count.

Abstrak. Latihan fisik intensitas tinggi dapat menyebabkan timbulnya stres fisik yang akan menekan sistem imun pada tubuh atlet. Penurunan fungsi sistem imun tersebut akan meningkatkan risiko infeksi dan menurunkan performa atlet. Pemberian diet dengan pengaturan indeks glikemik dan beban glikemik diketahui dapat membantu menstabilkan kadar glukosa darah saat latihan sehingga dapat mempertahankan fungsi sistem imun. Tujuan penelitian ini adalah untuk mengetahui perbedaan pengaruh pemberian diet beban glikemik rendah dan tinggi terhadap respon imun pada atlet sepak bola remaja. Desain penelitian ini adalah *quasi experimental pre-post group*, dilakukan pada 22 atlet sepak bola remaja usia 15-17 tahun di Sekolah Sepak Bola Terang Bangsa Semarang. Kelompok BG rendah diberikan makanan dengan kandungan BG 9,15; sedangkan kelompok BG tinggi mengandung BG 27,29. Pemberian diet diberikan sekali waktu di pagi hari dan 2 jam kemudian subjek melakukan latihan RAST (*Running-based Anaerobic Sprint Test*) untuk memicu stres fisik. Respon imun yang diukur yaitu jumlah leukosit dan hitung jenis leukosit (neutrofil, limfosit, monosit, dan eosinofil). Tidak ada perbedaan signifikan kadar glukosa darah, jumlah leukosit, dan hitung jenis leukosit antara kelompok BG rendah dengan BG tinggi (p>0,05). Diet BG rendah menyebabkan peningkatan kadar glukosa darah dan jumlah leukosit yang lebih kecil setelah latihan daripada diet BG tinggi.

Kata Kunci : diet beban glikemik, respon imun, leukosit, hitung jenis leukosit.

INTRODUCTION

Football is a high-intensity endurance sport that lasts for 90 minutes (Kirkendall, 2011). High intensity exercise can cause physical stress and lead to immunodepression in athlete's body (Gleeson, 2007; Gunzer, Konrad and Pail, 2012). Decreased immune function result in increase risk of infection in athletes so that it can

interfere the recovery process and reduce performance during the next competition or training (Gunzer, Konrad and Pail, 2012). Immune responses that occur due to physical exercise are increase of leukocytes, neutrophils, monocytes, lymphocytes and natural killer cells (Maughan and Gleeson, 2010). Previous study have reported that leukocytes, neutrophils, lymphocytes, eosinophils, monocytes, basophils increased after high intensity exercise (75% Maximum heart rate) (Abdossaleh *et al.*, 2014). Impaired immune function because of physical exercise caused by an increase levels of stress hormones such as cortisol and catecholamine (Gleeson, 2007; Maughan and Gleeson, 2010). Increased cortisol not only occurs during exercise with high intensity but also depends on blood glucose availability. Low blood glucose will increase blood cortisol levels (Nieman, 2008).

The strategy of providing good nutrition to athletes can maintain immune system condition (Gunzer, Konrad and Pail, 2012). One of the nutrients that is important to maintain the immune system is carbohydrate (Karacabey and Ozdemir, 2012). Providing of appropriate carbohydrates will maintain the availability of blood glucose during training or competition (Burke *et al.*, 2011). Sufficient carbohydrate availability and stable blood glucose levels can reduce stress hormone responses, provide glucose as an energy substrate for immune cells and maintain immune system (Gleeson, 2006). Adequate intake of carbohydrates is a major factor to maintain athlete performance (Gunzer, Konrad and Pail, 2012).

In recent years athletes have been introduced to provide carbohydrates by regulating the glycemic index. Glycemic index (GI) is a number that shows the increase potential of blood glucose from carbohydrate contents in a food (Beavers, Kristen M., Leutholtz, 2008). Previous studies have shown that consumption of high carbohydrates foods with low GI can maintain blood glucose stability during exercise (Wong *et al*., 2008). The stability of blood glucose can reduce levels of stress hormones such as cortisol, so that the immune system is maintained (Nieman, 2008). The glycemic index only indicates the type of carbohydrate, without considering the total amount of carbohydrate contents in a food, which can also have an impact on blood glucose (Beavers, Kristen M., Leutholtz, 2008). The GI is perfected by the glycemic load (GL). GL is the amount of carbohydrate and IG in a food, which will affect the blood glucose level after consuming it (Augustin *et al.*, 2015). GL can more accurately describe the effect of food on blood glucose levels (Beavers, Kristen M., Leutholtz, 2008). A study conducted on adolescent soccer athletes showed that consumption low GI and low GL diet 2 hours before exercise will stimulate smaller metabolic changes, it causes blood glucose are more stable during exercise (Siwi, Dieny and Fitranti, 2017).

Previous studies aimed to examine the effect of GI and GL diets on immune responses in running athletes. As a result, consumption low GI and a low GL diet 2 hours before exercise causes smaller changes in leukocytes, neutrophils, and lymphocytes than consumption high GI and low GL diet (Chen *et al.*, 2008). Other studies stated that there is no effect of consumption carbohydrate-containing foods with diffetence of GI and GL 2 hours before exercise on immune function (Li, 2015). The immune response is influenced not only by nutrient intake, but also by age, sex, stress conditions (physical and psychological stress), sleep disturbance, duration, frequency, and intensity of exercise (Gleeson, 2006; Walsh, 2018). This study will analyze the difference effect of high glycemic load diet and low glycemic load diet 2 hours before exercise on leukocytes and leukocytes differential count (neutrophils, lymphocytes, eosinophils, monocytes).

METHOD

This research was conducted at the Terang Bangsa Semarang Football School. This research was a quasi experimental pre-post-test group design with 2 treatment groups. Each group consisted of 11 people chosen through simple random sampling. The inclusion criteria of subjects was men aged 15-18 years, join in football club for at least 1 year, minimum follow 90% attendance of physical training in the last 12 weeks, not consume carbohydrate-based supplements or sports drinks 24 hours before the study, not smoking and consume alcohol, not being sick, injured, or in the care of a doctor, not do high intensity physical exercise 24 hours before the intervention.

The subjects in this study were divided into two groups: group 1 was given a low GL diet and group 2 was given a high GL diet. The low GL group was given carbohydrate-source foods with a glycemic load of 9.15, while the high GL group was given carbohydrate-source foods with a glycemic load of 27.29 (TABLE 1). Diet was given once at 2 hours before training. Two hours after eating GL diet, subjects did RAST (The Running based Anaerobic Sprint Test) exercise to trigger physical stress in athlete's body. RAST method is performed by

subjects being asked to six times sprints run on a 35 meter straight track with maximum speed with a 10-second pause between each repitition.

TABLE 1. Nutritional Composition of Preexercise Meals

	High GL (Medium GI – High GL/ M-H)	Low GL (Low GI – Low GL/ L-L)
Calorie	391,53 kkal	400,18 kkal
Carbohydrate	45,57 g (47%)	28,77 g (30%)
Fat	19,47 g (45%)	20,66 g (46%)
Protein	9,09 g (8%)	23,56 g (24%)
Estimated mix GI	59,86	31,79
Estimated mix GL	27,29	9,15
Food contents	Boiled corn (100 g)	Boiled spaghetti (90 g)
	Sweetened condensed milk (40 g)	Srambled egg (50 g)
	Cheddar cheese (20 g)	Corned beef (25 g)
	Butter (10 g)	Palm oil (10 g)

Subject's characteristics data including name, age, and date of birth were taken using a questionnaire. Body Mass Index (BMI) data were measure using the Bioelectric Impedance Analyzer (BIA). Physical activity data was measured using the Physical Activity Level (PAL) form. Data of the last 24-hour meal intake was measured using a 24-hour recall form. VO_2max data was measured using a bleep test. Data on stress conditions (psychological stress) athletes were measured using the The Perceived Stress Scale (PSS) questionnaire. Sleep quality data were measured using the Pittsburgh Sleep Quality Index (PSQI) questionnaire. All this data was taken the day before the study was conducted.

Blood samples were taken to measure blood glucose levels, total leukocytes, leukocytes and differential count. Blood samples were taken 3 times, those are before the intervention, immediately after the RAST, and 1 hour after the RAST. Blood glucose levels are measured using a glucometer from a peripheral blood vessels at the fingertips. Blood samples from veins were taken to measure the total leukocytes and leukocytes differential count, then the blood samples was analyzed in the laboratory.

Statistical analysis in this study was using the independent-t-test if the data was normally distributed and Mann-Whitney test if the data was not normally distributed. The statistical analysis was used to examine the difference of blood glucose level, total leukocytes, leukocytes differential count, and other variables before the intervention, immediately after the RAST, and 1 hour after the RAST between two groups.

RESULT AND DISCUSSION

Total 22 adolescent football athletes from the Terang Bangsa Football School (SSB) were the subjects in this study with an average age of 16 years old. Data on subject characteristics consisted of age, body mass index (BMI), VO_2max, hemoglobin level, physical activity, stress conditions, and sleep quality (TABLE 2). There were no significant differences in all subject characteristics (p>0.05) between low GL and high GL groups.

TABLE 2 Subject Characteristics of Two Groups

Subject Characteristics	Low GL (n=11)			High GL (n=11)			p
	Mean±SD	Min	Max	Mean±SD	Min	Max	
Age (year)	16,00±0,77	15	17	15,91±0,83	15	17	0,78[b]
BMI (kg/m²)	21,02±1,64	18,40	23,50	22,84±2,49	19,10	26,30	0,06[a]
VO_2max (ml/kg/minutes)	48,93±3,58	44,65	57,46	45,80±4,84	37,10	54,10	0,09[a]
Physical activity (kcal/hour)	1,54±0,25	1,24	1,88	1,53±0,25	1,13	1,88	0,85[a]
Stress condition (score)	6,55±2.02	3	10	7,27±2,05	4	11	0,41[a]
Sleep quality (score)	15,18±3,60	8	19	15,91±4,16	10	22	0,67[a]

[a]Independent-t-test, [b]Mann-Whitney

Adequacy level of nutrient intake including energy, carbohydrate, fat, protein, vitamin A, B6, C, E, zinc and iron intake showed no significant differences between two groups (p>0.05) (TABLE 3). Energy, carbohydrate, and protein intake of the two groups lower than their needs (<80%). However, mean value of carbohydrate intake level was higher in the low GL group than the high GL group.

TABLE 3. Adequacy Level of Nutrient Intake in Both Groups

Adequacy Level of Nutrient Intake	Low GL (n=11)			High GL (n=11)			p
	Mean±SD	Min	Max	Mean±SD	Min	Max	
Energy intake (%)	75,78±23,93	44,92	116,67	75,19±21,78	41,63	116,82	0,95[a]
Carbohydrate intake (%)	67,27±20,78	36,38	106,79	62,91±16,69	31,43	91,07	0,59[a]
Fat intake (%)	96,37±33,74	48,69	153,41	98,7±24,37	65,01	132,92	0,86[a]
Protein intake (%)	73,93±28,55	32,99	133,58	73,85±22,73	40,08	120,41	0,99[a]
Vitamin A intake (%)	301,75±101,17	132	488,87	339,93±70,72	212,7	478,7	0,32[a]
Vitamin B6 intake (%)	79,02±26,89	46,15	138,46	88,11±20,46	61,54	123,08	0,38[a]
Vitamin C intake (%)	22,9±28,79	0,67	89,89	15,31±19,08	0	65,22	0,67[b]
Vitamin E intake (%)	0,36±1,00	0	3,33	0,48±1,61	0	5,33	0,62[b]
Zinc intake (%)	50,16±18,32	27,65	87,06	53,2±9,82	37,65	66,47	0,61[a]
Iron intake (%)	50,42±21,58	29,33	103,33	53,39±9,66	40	70	0,24[b]

[a]Independent-t-test, [b]Mann-Whitney

Mean value of blood glucose levels before the intervention were 95.09 mg/dL in the low GL group and 89.55 mg/dL in the high GL group. There were no significant differences in blood glucose levels before intervention between the two groups (p>0.05) (TABLE 4). This shows that the condition of two groups before intervention was in the same condition.

TABLE 4. Blood Glucose Level in Both Groups according to Three Times

Type of Diet	Blood Glucose Level (Mean±SD)		
	Before intervention	Immediately after exercise	1 hour after exercise
Low GL	95,09±15,93	97,00±14,18	87,55±6,23
High GL	89,55±8,42	93,64±9,55	87,00±6,96
p	0,32[a]	0,52[a]	0,85[a]

[a]Independent-t-test

This study showed no significant differences in blood glucose levels immediately after RAST exercise test and 1 hour after RAST exercise test (recovery period) between low GL dan high GL groups (p>0.05). Previous study also showed the same results that there was no difference in blood glucose levels immediately after 2400 meters running between the low GI-low GL group and the low GI-high GL group (Siwi, Dieny and Fitranti, 2017). That might happen because carbohydrate content in this study was too low, only 30% in the low GL group (TABLE 1). Other study have suggested that consuming high carbohydrate diet with low GI can increase muscle glycogen stores and provide fuel for high intensity exercise (Little *et al*., 2010). Previous study have shown that consuming low GI-low GL meal with a high carbohydrate content (66%) can reduce carbohydrate oxidation and provide the blood glucose during exercise (Chen, Wong and Wong, 2008). Low carbohydrate content in the low GL group may cause earlier remove muscle glycogen stores during RAST exercise and lead to increased carbohydrate oxidation.
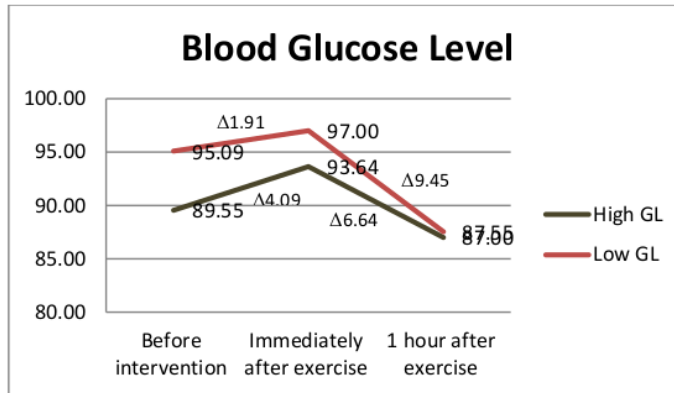
FIGURE 1. Graph of Change Blood Glucose Level in Both Groups According to Three Times

There was no difference in blood glucose levels at all three times between the two groups, but an increase of blood glucose levels from before intervention to immediately after exercise was lower in low GL group (Δ = 1.91 mg/dL) than high GL group (Δ = 4.09 mg/dL) (FIGURE 1). These results was similiar with previous studies which showed the increase of blood glucose levels in the low GI-low GL diet group occurred slowly for 2 hours after eating and during exercise (Chen, Wong and Wong, 2008). Decrease of blood glucose levels after 1 hour exercise (recovery periode) in the low and high GL group because the decrease of muscle glycogen immediately after exercise. Low carbohydrate content in diet of both groups (<50%) causes the use of blood glucose for glycogen re-synthesis and causes blood glucose decrease in both groups during recovery periode. (FIGURE 1) (Rollo, 2014).

TABLE 5. Total Leukocytes And Leukocytes Differential Count in Both Groups according to Three Times

Variable	Mean±SD		p
	Low GL Diet	High GL Diet	
Total Leukocytes			
Before intervention	7,37±1,28	7,32±1,94	0,95[a]
Immediately after exercise	10,07±1,01	11,17±3,91	0,67[b]
1 hour after exercise	6,98±1,02	8,12±2,83	0,25[b]
p	0,00[c]	0,00[d]	
Neutrophils			
Before intervention	50,55±5,65	55,09±8,75	0,16[a]
Immediately after exercise	44,73±9,37	49,18±11,33	0,33[a]
1 hour after exercise	55,59±5,79	63,09±8,51	0,03[a]
p	0,00[c]	0,00[c]	
Lymphocytes			
Before intervention	39,9±6,56	33,82±7,48	0,05[b]
Immediately after exercise	46,00±9,12	40,82±10,30	0,23[a]
1 hour after exercise	35,55±5,39	28,00±7.00	0,01[a]
p	0,00[c]	0,01[d]	
Monocytes			
Before intervention	7,45±1,44	8,09±2.47	0,71[b]
Immediately after exercise	7,82±1,17	8,09±1.76	0,81[b]
1 hour after exercise	7,09±1,14	7,64±2.42	0,84[b]
p	0,03[d]	0,27[d]	
Eosinophils			
Before intervention	2,09±1,04	3,00±1.41	0,10[a]
Immediately after exercise	1,45±0,69	1,82±0.87	0,26[b]
1 hour after exercise	1,45±1,04	1,27±0,90	0,78[b]
p	0,00[d]	0,00[d]	

[a]Independent-t-test, [b]Mann-Whitney, [c]Repeated ANOVA, [d]Friedman

Total leukocytes and leukocytes differential count (neutrophils, lymphocytes, monocytes, eosinophils) at all three times between low GL and high GL group showed no significant difference (p>0.05), except neutrophils and lymphocytes 1 hour after exercise showed significant difference between two groups (p<0.05) (TABLE 5). These results differ from previous studies which showed that there were significant differences total leukocytes and neutrophils between the low GI-low GL diet group and high GI-low GL diet group (Chen *et al.*, 2008). There was no difference between two groups in this study probably because of differences physical stress exposure from the exercise compared to previous study. Exercises in previous study was constant running at 70% VO_2max for 1 hour and then followed by 10 km running (Chen *et al.*, 2008). So, besides the intensity of the exercise, the duration of the exercise also affects changes in the body's immune system (Terra *et al.*, 2012; Palmowski *et al.*, 2019). Impaired immune function after exercise is greater when exercise occurs continuously and prolonged (1.5 hours), with moderate to high intensity (VO_2max 55-75%) (Gleeson, 2007). Long duration intensive exercises such as marathon or ultramarathon can suppress immune system function (Palmowski *et al.*, 2019).

Carbohydrate intake of subjects 1 day before intervention were less than normal requirements in both groups. This may be another reason there is no difference in total leukocytes between two groups. Low carbohydrate intake causes a lack of muscle and liver glycogen stores. Lack of glycogen stores can reduce the rate of ATP regeneration and release of Ca^{2+} in the sarcoplasmic reticulum during exercise, so the muscles are not able to provide enough energy for muscle contraction during exercise (Ortenblad, Wasterblad and Nielsen, 2013). Adequate carbohydrate intake can increase the availability of blood glucose during exercise and prevent an increase cortisol levels so the function of immune cells is maintained (Ziaolhagh and Naghibi, 2012).
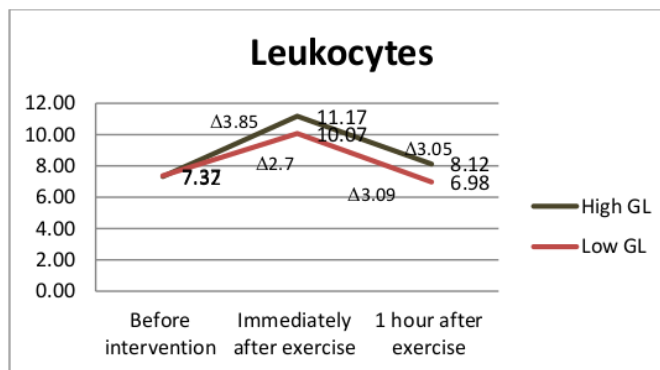

FIGURE 2. Graph of Change Total Leukocytes in Both Groups According to Three Times

This study showed that an increase of total leukocytes before intervention and immediately after exercise between two groups. That was similiar with previous study, there was an increase of total leukocytes after 10 km running on subjects given GI and GL diets (Chen *et al.*, 2008). Other study also showed an increase of total leukocytes after playing football and running. This happens because high intensity exercise produces high physical stress, causing changes in total leukocytes (Cenikli, 2016).

Although total leukocytes at three times showed no difference between low GL and high GL groups, the increase of total leukocytes before intervention to immediately after exercise was lower in the low GL group ($\Delta = 2.7$x10^3 µL) than high GL group ($\Delta = 3.85$x10^3 µL) (FIGURE 2). That was probably because the diet in the low GL group contained low GI food. Consumption of foods with low GI before exercise has more potential to maintain the availability of blood glucose during exercise (Wong *et al.*, 2008; Little *et al.*, 2010). Stable blood glucose can prevent the increase in stress hormones and maintain immune system function. (Nieman, 2008; Gleeson, Bishop and Walsh, 2013).
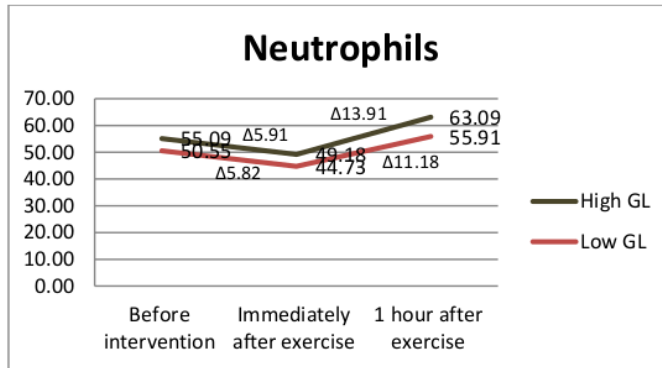
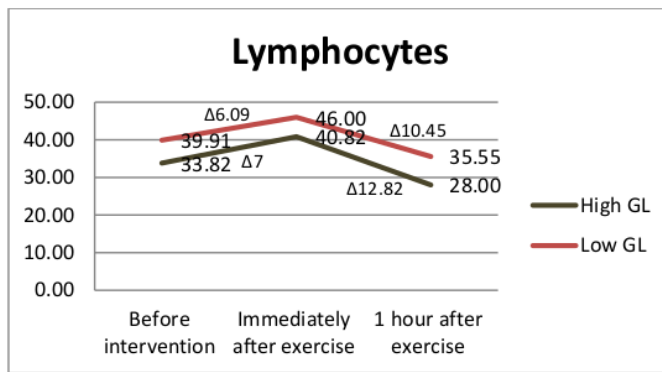**FIGURE 3. Graph of Change Neutrophils in Both Groups According to Three Times**

FIGURE 4. Graph of Change Lymphocytes in Both Groups According to Three Times
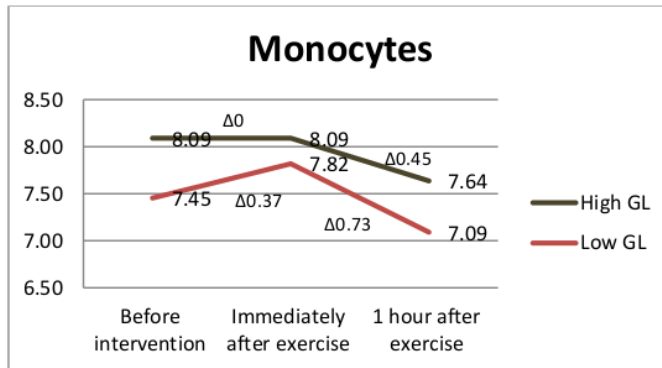

FIGURE 5. Graph of Change Monocytes in Both Groups According to Three Times
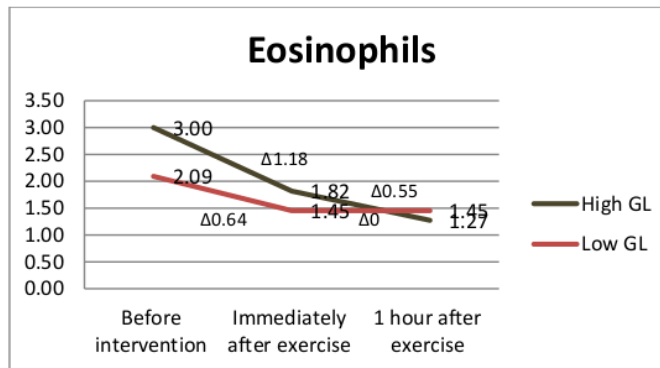
FIGURE 6. Graph of Change Eosinophils in Both Groups According to Three Times

Changes in total leukocytes and leukocytes differential count can occur due to the short-term (acute) effects of high-intensity exercise. These changes occurred immediately after exercise until 2 hours after exercise (Neves *et al.*, 2015). In this study an increase of leukocytes, lymphocytes, monocytes, and a decrease of neutrophils and eosinophils after exercise between both groups. Previous studies have shown that there was an increase in leukocytes, neutrophils, lymphocytes, and monocytes immediately after 10 km running on subjects who were given a carbohydrate diet with the regulation of GI and GL (Chen *et al.*, 2008; Li, 2015).

The increase in leukocytes (leukocytosis) due to acute exercise is temporary because the amount will return to the resting value 6-24 hours after exercise. Leukocytosis from physical exercise is caused by mobilization or changes in the number of neutrophils and lymphocytes, as well as the small contribution of monocytes. High-intensity exercise that lasts briefly (several minutes) can cause an increase in neutrophils to 2 times more, while endurance training that lasts long causes an increase in neutrophils to 3-4 times more (Gleeson, Bishop and Walsh, 2013).

There are several limitations in this study, such as no data about muscle glycogen stores and blood cortisol levels. Muscle glycogen will affect blood glucose levels during exercise so it will affect the hormone cortisol and cause changes in the immune system. In addition, this study did not directly test the glycemic index of food on blood glucose levels, only through the calculation of various references. Even though there is no standard for the glycemic index of food so the values are different and make the glycemic load of food also different.

CONCLUSION

There was no difference effect of high and low glycemic load diets 2 hours before Running-based Anaerobic Sprint Test (RAST) on the immune response of adolescent football athletes characterized by no differences in total leukocytes and leukocyte differential count (neutrophils, lymphocytes, monocytes, and eosinophils). However, increases in blood glucose levels and total leukocytes after RAST were lower in the low GL diet group. High-carbohydrate diets (> 60%) with low GI and low GL can be used to high intensity exercise.

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