Three Weeks of High-Intensity Interval Training (HIIT) Decreases Visfatin Level on Overweight Men

by Lilik Herawati

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Malaysia

Daniel John Green
University of Western Australia
Australia

Fadzil Hamzah
Sport Center of Changi General Hospital
Singapore

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Misbakhul Munir, Universitas Airlangga, Indonesia

FOREWORD

Dean of Faculty of Medicine, Universitas Airlangga

Assalamu'alaikum Wr. Wb.

Distinguished Guests, all the Participants, Ladies and Gentlemen

On behalf of Faculty of Medicine, Universitas Airlangga, it is my great pleasure to welcome all the speakers, moderators, and participants on **Surabaya International Physiology Seminar 2017 (SIPS 2017)**, which will be held from today, October 12th until October 14th, 2017. I would like to express my hearty welcome to all the international speakers, **Prof. Cheng Hwee Ming**, from University of Malaya, Malaysia; **Prof. Daniel John Green**, from University of Western Australia; **Dr. Fadzil Hamzah**, from Sport Center of Changi General Hospital, Singapore and **Dr. Deanne Helena Skelly**, from Griffith University, Australia.

The aim of SIPS 2017 is to provide a platform for academicians, educators, researchers, practitioners, undergraduate and postgraduate students to share and discuss the knowledge of the recent issues, opinions, researchers about the development and innovation of physiology in medical science, dentistry, veterinary, plants and agriculture, sports and sciences.

I believe this event is a great purpose in order to develop knowledge, experiences and best practices that can be applied for the good, especially in the field of healthcare as a whole.

Finally, I would like to express my sincere acknowledgements to those who take part and especially for Department of Medical Physiology, Faculty of Medicine, Universitas Airlangga for their effort in holding this event and wishing all to have success.

Wassalamu'alaikum Wr. Wb.

Prof. Dr. Soetojo, MD.
Faculty of Medicine, Universitas Airlangga

Chair of Committee / Head of Physiology Department, Faculty of Medicine, Universitas Airlangga

Assalamu 'alaikum Wr. Wb

Greetings,

On behalf of SIPS committee and Physiology Department, Universitas Airlangga, we are welcoming to Surabaya, City of Heroes.

This year, the annual meeting of Indonesian Physiology Society (IAIFI) is hosted at Surabaya, entitled **"Surabaya International Physiology Seminar Workshop (SIPS)"**. We present some update workshop and lectures in order to bring physiology research from basic to clinical application on humanities, animal welfare and good environment. All participants have opportunities to publish their research in presentation, poster and ISBN proceeding. Selected papers will be submitted to SCOPUS indexed proceeding/ journal and awarded as Best Poster and Best Oral Presentation.

We hope that all participants will get some interesting experiences for next 3 days, 12-14 October 2017. Enjoy our lectures and workshops, taste the culinary and take your time to sightseeing around Surabaya.

Wassalamu 'alaikum wr. wb.

Dr. Bambang Purwanto
Chairman of Committee / Head of Physiology Department
Faculty of Medicine, Universitas Airlangga

Welcome Address - Surabaya International Physiology Seminar Workshop (SIPS)

Dear fellow Physiologists and Participants,

On Behalf of the Indonesian Physiological Society (IAIFI) and the Physiology Department Faculty of Medicine Universitas Airlangga, I would like to welcome you all to Surabaya International Physiology Seminar (SIPS), held on 12-14 of October 2017.

Finally after long-awaited Surabaya gets a turn again to host and organize the International Physiology Seminar. Hence the Steering– and Organizing Committee consisting of young energic physiologists are determined to make the Seminar a successful one. The theme of the seminar is:

"The Role of Physiology in Translation Research: From Basic to Application"

This annual meeting covers a wide range of topics of Physiology on Medicine, Dentistry, Veterinary, Plants and Agriculture, Sports and Sciences. We sincerely hope that SIPS 2017 enable to provide a platform for academicians, educators, researchers, practitioners and postgraduate students to present and discuss researches, development and innovations in wide range of topics as mentioned above. It will provide all participants to share knowledge, exchange new ideas and their experiences in many research topics, for then it will enhance future collaborations.

With great interest and enthusiasm I look towards the success of this Seminar, and wish all of you every success and a pleasant stay in Surabaya.

May Allah Swt. bestow upon us His Blessings.

On Behalf of the Steering and Organizing Committee Senior Physiologist,
Prof. R. Soedarso Djojonegoro

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PAPERS

FULL PAPERS

Intermittent Physical Training Decreases Peak of Blood Glucose Level after Meals in Rats

Eka Arum Cahyaning Putri, Raden Argarini, Bambang Purwanto and Lilik Herawati
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Keywords: Blood Glucose, Exercise Intensity, Physical Activity.

Abstract: Some people prefer doing exercise following the intermittent model while others prefer the continuous one . However, it is still unclear which is the best one for blood glucose regulation. This study was carried out to determine the difference between the changes in blood glucose levels after doing physical exercise for the intermittent and the continuous model. The subject was male adult rats divided into 3 groups: control, continuous, and intermittent, 5 rats in each group. The rats swam in moderate intensity every day for 8 weeks. The results showed that the control group had the highest results of the peak (30 minutes) blood glucose levels after meals followed by the other 2 groups The results of the intermittent group had a significant peak in blood glucose levels 30 minutes after meals ($p < 0.05$). Also the differences in blood glucose 30 minutes and 60 minutes after meals in the intermittent and continuous groups were significantly different than the control group ($p < 0.05$). During the 8 weeks of moderate physical exercise every day, it can be assumed that there was more insulin secretion to reduce peak blood glucose levels after meals. The lower blood glucose difference at 30 minutes and 60 minutes after meals of both the continuous and intermittent groups compared with the control group indicates that glucose uptake by cells is better in those groups . In conclusion, our data support the benefit of intermittent and continuous exercise training for the optimal regulation of blood glucose levels. The intermittent model also has more effect on the peak phase of blood glucose level after meals.

1 INTRODUCTION

Physical exercise is one way to regulate blood glucose levels. This is known through the improvement of GLUT-4 translocation in skeletal muscle when exercise increases glucose uptake by cells. According to the exercise and rest phase, physical exercise can be done intermittently and continuously. Continuous physical exercise means the exercise is done continuously and rest taken at the end of the exercise, while intermittent physical exercise is done with periods of exercise alternating with periods of rest. When observed, people generally do physical exercise with intermittent models (Shepherd, 1999; Ganong, 2001).

It is known that increasing the GLUT-4 translocation in skeletal muscle is important because it can indirectly result in the balance of blood glucose levels. Previous research has discovered that physical exercise can affect blood glucose levels by a mechanism independent of insulin. Stimulating this mechanism may be an alternative for example for people with diabetes mellitus, in regulating blood glucose levels.

It is also known that intermittent and continuous submaximal physical exercise decreases blood glucose levels (Herawati, 2004). It can be assumed that there has been an increase in glucose uptake by cells through enhancement of GLUT-4 translocation, but how much improvement in GLUT-4 translocation in physical exercise of intermittent and continuous submaximal models remains unknown (Ganong, 2001; Zierath, 2000)

From Herawati's (2004) study comparing acute physical exercise on an intermittent and continuous basis, there was a noticeable decrease in glucose levels during physical exercise, but during recovery, the control group experienced a decrease in glucose levels.

According to the description above, this research was designed to find out the effect of a training program of moderate intensity physical exercise

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with an intermittent and a continuous model on the speed of glucose uptake in skeletal muscle.

2 METHODS

This research is true experimental laboratory with random control posttest-only design. The population in this study were male white rats aged 2-3 months. The sample size was 5 heads per group, divided into 3 groups: control, continuous, and intermittent. The division of groups was random sample using the lottery method. This research was conducted in the Biochemistry Laboratory of Faculty of Medicine Universitas Airlangga, Surabaya.

The independent variable of this study was a medium-intensity physical exercise program in an intermittent and a continuous model every day for 8 weeks. In the intermittent training program, the rats were moderated by physical exercise of moderate intensity, ie 5% Body Weight load for maximum time that could be achieved but performed alternately between swimming and rest with a 2:1 ratio. For example, the work time in the pool was 30 seconds then the rest period was 60 seconds. In the continuous intensity physical exercise program, the rats were reinforced with submaximal intensity exercise, ie 5% BB load for the maximum time that could be achieved.

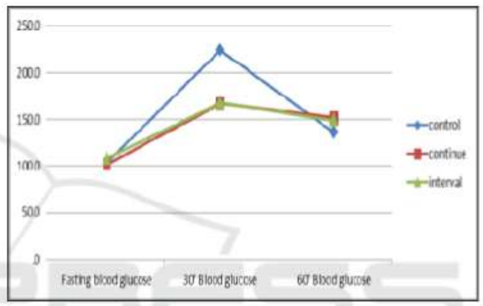
Variables dependent on this study were serial blood glucose levels examined with an Accu-Chek glucosemeter from Roche. Serial blood glucose levels were examined by cutting the male white rat's tail to obtain the drops of blood. The first drop of blood was removed, then a drop of blood after it was absorbed into a test strip, waiting 15 seconds until the blood glucose level appeared on the screen while the blood drops were stopped by pressing the bleeding site. The number or value shown is in new units of mg/dl.

Blood glucose levels examined were fasting blood glucose levels, 30 minutes and 60 minutes after an intra-peritoneal glucose tolerance test. In addition, changes in blood glucose levels were also calculated by measuring the difference in fasting blood glucose levels and 30 minutes, the difference in blood glucose levels between 30 minutes and 60 minutes, and the difference in fasting blood glucose levels and 60 minutes.

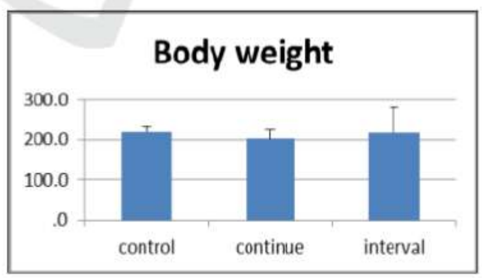
At the end of the 8th week, there was a 2-day pause followed by statistical analysis and the production of descriptive statistics, a distribution normality test, a homogeneity test, and an ANOVA test if the data were normally distributed, or a Kruskal-Wallis test if the data were not normally distributed.

3 RESULTS

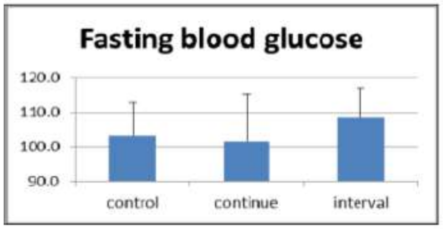
Overall, the 3 groups had nearly equal data of fasting blood glucose. The 3 groups also experienced elevated blood glucose 30 minutes after meals compared with fasting blood glucose. In the control group, blood glucose 30 minutes after meals was much higher in value compared to the other 2 groups. Then, in the blood glucose examination 60 minutes after meals, all 3 decreased with values that were not much different.



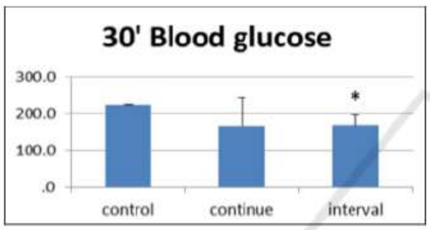
The number of white rats that were used in this study was 15 and these were divided into 3 groups (control, continue, and interval). Overall, the rats had an average body weight that did not differ between groups. This shows that body weight was not a confounding factor in this study. Likewise the type and age of animals used were not different.



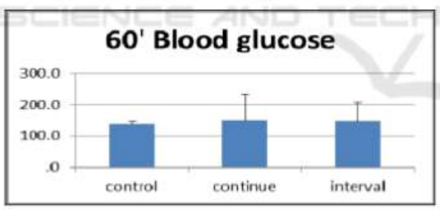
Fasting blood glucose examination is shown on the graph below. The intermittent group had the highest yield, and the continuous group had the lowest result. But the results of fasting blood glucose examination of the 3 groups were still in the range of values that were not different.



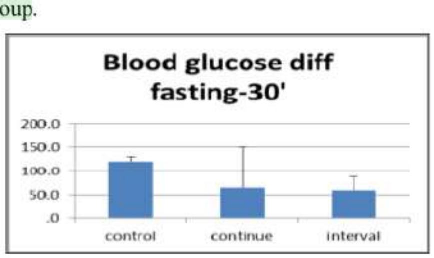
Thirty minutes after meals (using the peritoneal D40 glucose fluid), the control group had the highest yield. This was followed by the intermittent group, and the continuous group had the lowest yield. The result of the intermittent group showed a significantly different blood glucose level 30 minutes after meals ($p < 0.05$).



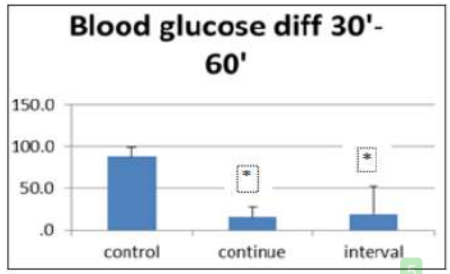
On examination of blood glucose 60 minutes after meals, the intermittent and continuous groups had similar results while the control group had slightly lower results.



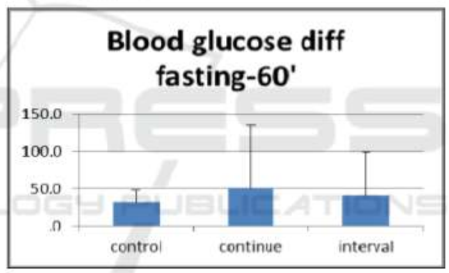
The graph below shows the difference between fasting blood glucose and blood glucose 30 minutes after meals. The control group had a difference in fasting blood glucose and blood glucose 30 minutes after meals, followed by the continuous group, these did not differ significantly with the intermittent group.



In the graph below, the largest difference between blood glucose 30 minutes and 60 minutes after meals of the 3 groups was the control group, followed by the intermittent and continuous groups. The results of the intermittent and continuous groups were significantly different from that of the control group.



The graph below shows the difference in fasting blood glucose and blood glucose 60 minutes after meals. In all 3 groups, there were no significantly different outcomes.



4 DISCUSSION

Guyton mentioned a glucose tolerance curve: when a normal person who is fasting then consumes 1 gram of glucose per kilogram of body weight, his blood glucose level will rise from about 90 mg/dl to 120-140 mg/dl, and within approximately 2 hours this level will go down again to its normal value (Guyton, 2008). However, with diabetic patients, Guyton also mentioned that fasting blood glucose concentration is almost always above 110mg/dl. This was consistent with significantly different results ($p < 0.05$) in the blood glucose levels obtained 30 minutes post-intermittent exercise by glucose tolerance test compared with the other 2 groups. Physical activity performed at intervals daily for 8 weeks stimulates better insulin secretion thereby reducing peak blood glucose levels after meals.

The result of decreasing peak blood glucose levels after a meal benefits the body because Guyton mentions that blood glucose levels need to be kept from rising too high even after eating. One of the dangers when blood glucose concentration rises too high is that glucose can cause large amounts of osmotic pressure in the extracellular fluid and may cause cell dehydration.

Herawati (2004) mentions that submaximal intermittent and continuous physical exercise decreases blood glucose levels. The blood glucose levels in question are those 30 minutes and 60 minutes after meals. In this study, blood glucose levels of 30 minutes and 60 minutes after meals also showed significant differences ($p < 0.05$) in the continuous and intermittent groups. This is when compared with control group who did not perform physical activity.

The lower blood glucose difference of 30 minutes and 60 minutes after meals in the continuous group indicates glucose entry into cells was slower. This is good for diabetic patients because it is expected that blood sugar levels after eating or drinking will be increased lightly but gradually. This can stimulate the pancreas to produce insulin so as to prevent the rise in blood sugar levels further and cause blood sugar levels to decline slowly.

5 CONCLUSIONS

Based on the results of the study and discussion it can be concluded that: (1) physical exercise at intervals with moderate intensity can decrease peak blood sugar level; and (2) there is a significant difference between the control group who did not do physical exercise and the intermittent and continuous groups who were doing physical exercise in keeping blood glucose levels lower after meals. There were decreased blood glucose levels between 30 minutes and 60 minutes after eating in both groups doing physical exercise (intermittent and continuous).

Although the results of this study have provided additional information about the physiological effect of physical exercise on blood glucose levels, further research is needed, both to extend theoretical explanations and their application.

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Non-Invasive Method on Slow-Twitch Quadriceps Muscle Fibers Dominate a High Level of Fitness

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Keywords: Fitness Level, Muscle Fiber Type, Non-Invasive Method, VO_2max.

Abstract: Previous studies have proved that there is a linear correlation between VO_2max and slow-twitch fibers in athletes. These studies were done using biopsy, the invasive method, to estimate the muscle fiber type. There is also a non-invasive method, which is convenient to use and not conflicted by ethical law in Indonesia. However, further study regarding muscle fiber shifting and its correlation with the level of fitness using a non-invasive method to estimate muscle fiber is still lacking. This research aims to determine the distribution and correlation between level of fitness and muscle fiber type in non-athletes estimated by a non-invasive method. Muscle fiber type in the quadriceps muscle group and level of fitness were determined in 33 untrained male students with an average age of 20.5 y.o. Muscle fiber type was determined by a non-invasive method by counting the maximal repetition with 80% 1RM weight. The level of fitness was determined a week later by the *Astrand ergocycle* method. The slow-twitch muscle fibers dominated a high fitness level ($p=0.002$). However, the correlation test between muscle fiber types and fitness level was not significant ($p=0.551$). This result showed that a high fitness level, which has a higher oxygen consumption, has numerous slow-twitch muscle fibers. It is rich in myoglobin delivering oxygen to maintain the aerobic activity. Yet there is no correlation between the level of fitness and muscle fiber type as estimated by a non-invasive method in quadriceps. Further studies are needed to analyze several factors that may contribute to it.

1 INTRODUCTION

Physical fitness has a direct relationship with the aerobic energy system, muscle ATP-PC capacities, and lactate metabolism (Robergs, 2003). Physical fitness can be determined by measuring VO_2max or maximal oxygen uptake (Ganong, 2010). VO_2max reflects the maximal amount of oxygen that has been consumed during intense physical activity.

Muscle fiber has been classified into slow-twitch (Type I) fiber and fast-twitch (Type II) fiber (Scott et al., 2001). The term mixed-twitch fiber has been used if there is an equal proportion of slow-twitch type and fast-twitch type in one muscle group. Slow-twitch fibers have a greater aerobic capacity, larger vascular and capillary, and are more resistant to fatigue than fast-twitch fibers (Ganong, 2010). Fast-twitch muscle was used for power and speed events due to its anaerobic capacity (Berning and Steen, 2005). Skeletal muscle consists of muscle tissues which can easily adapt due to the environment that change its constituent protein (Magaudda et al., 2004).

Several studies show that shifts in muscle fiber from fast-twitch fiber to slow-twitch fiber is possible due to long periods of endurance type events (Wilson et al., 2012). Athletes who are involved in long periods of endurance training have very high levels of VO_2max (Skinner, 2005) and a high percentage of slow-twitch fibers (Plowman, 2007). A previous study has proved that there is a linear correlation between VO_2max and slow-twitch fibers in athletes (Bergh et al., 1978). However, most of the research was carried out on athletes or trained men. Further study regarding muscle fiber shifting and its correlation with level of fitness in non-athletes and untrained men is still lacking. Moreover, a non-invasive method to identify muscle fiber type is still clearly unknown. The purpose of this study was to determine the distribution and

Ika Putri, Y., Triadi Desnantyo, A. and Herawati, L.
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correlation between level of fitness and muscle fiber type in non-athletes estimated by non-invasive method.

2 MATERIALS AND METHOD

Thirty-three untrained males aged 19–22 years participated in this study. Muscle fiber type in the *quadriceps* muscle group was determined by counting maximal repetition with 80% 1 RM weight during leg extension. This method is introduced and applied in muscle groups, not to an individual muscle (Kraps, 2001). Maximal repetition at a predetermined load and muscle fiber type have a fair to moderate relationship (Douris et al., 2006).

Level of fitness was determined using the *Astrand* method to measure predictive value of VO_2max while using the ergocycle. The *Astrand* method has been proven to be accurate in determining VO_2max in healthy young men and women (Hoehn et al., 2015). Then, the VO_2max was classified in low, average, high, and very high levels of fitness.

3 RESULTS

Frequency distribution of muscle fiber type of quadriceps muscle group among participants is presented at Fig. 1. Fifty-five percent participants have slow-twitch fibers, 39% mixed-twitch fibers, and 6% slow-twitch fibers. Frequency distribution of level of fitness among participants is presented at Fig. 2 where 21.2% participants have low level of fitness, 30.3% average, 33.3% high, and 15.2% very high.

The slow-twitch muscle fibers dominate low levels and high levels of fitness. There were five participants with slow-twitch and two participants with mixed-twitch muscle fibers at a low fitness level. In the high level of fitness, there were seven participants with slow-twitch and four participants with mixed-twitch muscle fibers. However, the significant difference between slow-twitch and mixed-twitch muscle fibers ($p=0.002$) was only in high level of fitness The participants with average level fitness were dominated by mixed-twitch muscle fibers, yet there was no significant difference. The fast-twitch muscle fiber type was detected in a small proportion ($p \geq 0.05$) on average and a very high level of fitness. The frequency of distribution based on classification of fitness level and muscle fiber type of participants is presented in Fig 3.

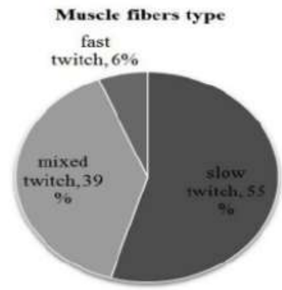

Figure 1: Frequency distribution of muscle fiber type of quadriceps muscle group (n=33).

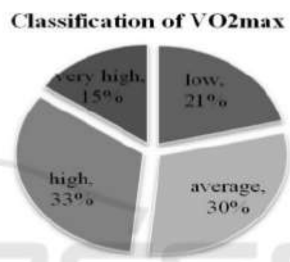

Figure 2: Frequency distribution of level of fitness (n=33).

P value was determined by the *Spearman* correlation test; p = 0.551 showed that there was no correlation between level of fitness and muscle fiber type (Table 1).

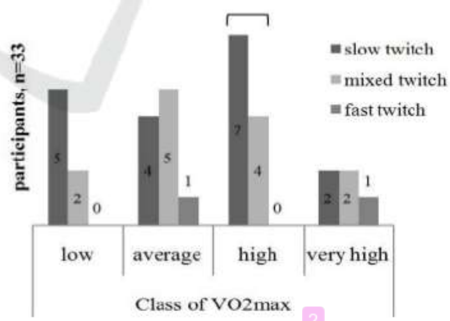

Figure 3: Frequency distribution of level of fitness on muscle fiber type.

*significant difference (p<0.05)) between slow and mixed-twitch muscle fibers.

Table 1. Correlation between level of fitness and muscle fiber type.

Spearman rho n=33	Classification of VO_2max	
Muscle fiber types	Correlation Coefficient	0.108
	Sig. (2-tailed)	0.551

4 DISCUSSION

The main finding in this study was that there is a significant difference between slow and mixed-twitch muscle fibers at a high level of fitness; however, there is no correlation between muscle fiber type and level of fitness in quadriceps of non-athletic young men. The domination of slow-twitch muscle fibers at a low and high fitness level is similar to the research by Barstow et al. (2000). Nine healthy participants were measured muscle fiber type recruitment based on the exercise intensity. The type I muscle (slow-twitch muscle fibers) was dominant in the low and/or high intensity of exercise (Barstow et al., 2000). Nevertheless, the correlation is contrary to the research from Barstow et al. (2000) and Bergh et al. (1978). One has to keep in mind that this study was done on untrained young men while their observation was done on athletes and trained men. Moreover, this study only uses *quadriceps* muscle to determine the muscle fiber type whereas the distribution between muscle fiber type varies in each muscle group.

There are some other factors that can influence level of fitness. Oxygen delivery is a primary factor in VO_2max limitation during exercises (Bassett and Howley, 2000). While athletes who have higher VO_2max also have a high percentage of slow-twitch fibers, VO_2max is not limited by the percentage of slow twitch muscle that someone is born with (Foss and Keteyian, 1998). Bergh's observation also found that VO_2max is higher in athletes than non-athletes in slow twitch fibers. This indicates that training and physical activities also influence VO_2max. Furthermore, studies regarding shift of muscle fiber from fast-twitch to slow twitch that was induced by training are still conflicted. It have already been cleared is muscle have the ability to alter its structural and functional properties to adapt to the environmental conditions imposed on it is known as muscle plasticity (Gransee et al., 2012). The mitogen-activated protein kinase signaling has been the possible pathway involved in exercise-induced adaptations in skeletal muscle (Hawley, 2002). Endurance exercise training can allegedly induce adaptive muscle fiber transformation and increase mitochondrial biogenesis (Wang et al., 2004). However, the endurance training procedure and period that can actually change the fast-twitch fiber to slow-twitch fiber are still being debated. The majority of evidence still suggests that cross-innervation is the only way to effectively change the fast-twitch fiber into a slow-twitch fiber (Foss and Keteyian, 1998).

5 CONCLUSION

In summary, a high level of fitness has slow-twitch muscle fibers type domination with the invasive method; however, the correlation between the level of fitness and muscle fiber type in quadriceps of young men has not been found in this study. Many other factors can influence level of fitness and muscle fiber type distribution. Factors and metabolic change pathways as well as the training procedure and period that can actually change the muscle fiber type from fast-twitch to slow-twitch are suggested as potential for further studies.

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High-Calorie Diet Reduces Neuroglia Count

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Keywords: Astrocyte, Cerebral Cortex Diet, High Calorie, Hyperglycemia.

Abstract: Nowadays, high-calorie food consumption people can unwittingly affect health. Several studies have reported the effects of excesshigh-calorie food consumption can range fromhyperglycaemia to neurodegenerative interference. In the brain, astrocytes function to respond to the modificationof molecular brain structure. This study aimed to observe the effect of a high-calorie diet on the brain histology of male mice (*Mus musculus*). In this study, 28 mice were randomly divided into 4 groups: the control group pre (K0); the control group post with a standard diet (K1) ;the treatment group with 0.15g of glucose diet (K2); and the treatment group with 0.25g of glucose diet (K3). Treatment was given everyday for eightweeks. Brains were then histologically processed and stained with Haematoxylin-Eosin (HE) and observation made of quantitative changes of astrocytes in the cerebral cortex. Results: Data were analyzed with Post-Hoc ANOVA. From the study, significant differences were found between K0 and K2 (α <0.05). In addition, significant differences were obtained in the groups K0 and K3 (α <0.05). Meanwhile, no significant differences were found between groups (α> 0.05). In conclusion, a high-calorie diet affects thebrain histology of male mice.

1 INTRODUCTION

Nowadays, high-calorie foods are consumed in high quantities by people without them realizing the that these types of food may result inbad habits. Eating excessive high-calorie foods can lead to metabolic diseases and cardiovascular disorders, even in the central nervous system (Auer *et al*., 2015). When the body receives an excessive intake of high-calorie foodslong term , this can lead to an increase in blood glucose levels. If this condition continues, it can cause hyperglycemia (Guyton & Hall, 2011). Hyperglycemia can lead to a decrease in the activity of insulin, which serves to increase glucose uptake in most tissue cells (CDC, 2011). In most cell tissues the body needs insulin for glucose uptake, but the insulin-free brain does not. At the time of hyperglycemia, where insulin insufficiency occurs, the brain will still obtain adequate nutrition, but with further consequences, the condition willeventually lead to brain dysfunction (Sherwood, 2014). Other tissues can produce adenosine triphosphate (ATP) without oxygen; however,ATP cannot occur in the brain because the brain requires oxygen to produce it. Another unique aspect of the brain compared to other tissues is that the brain in normal circumstances can only use glucose as an energy source but cannot store it. Therefore, the brain depends entirelyon adequate intake and a constant amount of glucose and oxygen. Brain damage might occur without sufficient intake of oxygen for more than fourto five minutes or if its glucose discharge is cut off from 10 to 15 minutes (Sherwood, 2014). Hyperglycemia can lead to excess superoxide production of mitochondria in the endothelial cells of the blood vessels. Increased production of superoxide may lead to increased activation of advanced glycogen end-products (AGEs/AGEP). This condition leads to the formation of intracellular reactive oxygen species (ROS) that can: cause angiogenesis defects in response to ischemia; activate a number of pro-inflammatory pathways; and cause epigenetic long-lasting changes that

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encourage persistent expression of pro inflammatory genes after hyperglycemic memory (Giacco & Brownlee, 2011). Changes in blood glucose levels affect the brain cells directly or indirectly because glucose is the main source of energy in addition to oxygen. A quarter of the body's oxygen is used for brain cells. This large energy-use brain cell is activated in the mitochondria, in order tomaintain braincellfunctional activity. In the brain, cells that play a major role in the neurotransmitter process and respond to biochemical changes are called astrocytes. Changes in blood glucose levels in the body can cause the intake of glucose and oxygen to the brain to be disrupted, meaning thatthe brain cannot run properly. Excessive consumption of a high-calorie diet can affect the body's metabolism, leading to hyperglycemia, which affects the brain tissue directly or indirectly, as described above. This has encouraged researchers to study the impact of a high-calorie diet on the histology of the brains of mice, and to increase publicawareness ofthe impact of a high-calorie diet upon the body, especially brain tissue.

2 METHODS

The protocol used in this study has already been approved by Ethical Committee, Faculty of Veterinary Medicine, Universitas Airlangga.

The standard diet ismouse food obtained from the Faculty of Veterinary Medicine, Universitas Airlangga, and provided with mineral water which can be accessed freely (ad libitum) and 0.5cc of mineral water by oral gavage per day. A high-glucose diet is a standard diet, and 0.15g and or 0.25g of glucose in 0.5cc solution by oral adminastrationwithout considering the weight and ad libitum glucose solution with a concentration of 0.05g/ml.

Twenty-eight mice were randomly divided into four groups. The first group (K0) is the pre-treatment control group. The second group (K1) is the standard dietary treatment group. The third group (K2) is a group with ahigh-glucose dietary treatment of 0.15g. The fourth group (K3) is a group with ahigh-glucose dietary treatment of 0.25g. The mice were adapted in a cage for 1 week prior to treatment. During the adaptation, they were fed with the standard food and drink ad libitum. The first group (K0) was sacrificed after a 1-week adaptation as pre group data. The other group received treatment for 8 weeks. Mice were treated as above, where glucose is administered by oral galvage once per day for 8 weeks. After being treated for 8 weeks, the mice were all sacrificed. Prior to being sacrificed, the mice were given anesthesia by intra-peritoneal injection. The head of the mice was dissected and the brain was taken out and then fixed with formalin buffer. The tissue was then stained using HE (Hematoxylin-Eosin) in the Histotechnic Laboratory of Anatomical Pathology Department of the Faculty of Veterinary Medicine Universitas Airlangga. Microscopic examination of brain tissue was conducted afterward. The astrocytes were then counted from 5 areas, which was at the top, right center, center, left center and bottom, and 400x magnification. Microscope mounted graticulae with 5x5 count chamber. Changes in the amount of astrocyte was counted and the result was analyzed statistically using SPSS.

3 RESULTS

Among 28 mice, there were 9 mice died during the study, 1 mouse in the post control group (K1), 3 mice in the glucose diet treatment group of 0.15g(K2) and 5 mice in the 0.25g glucose diet treatment group (K3). As of this, the amount of mice that performed histology of the brain is from 19 mice.

Table 1: Result of astrocyte count.

Group	N	Astrocytes (%) (*Mean* ± SD)
K0	7	18.43 ± 5.56
K1	6	16.00 ± 1.89
K2	4	12.75 ± 1.70
K3	2	10.00 ± 1.41

The least amount of astrocytes was obtained in the 0.25 gram (K3) glucose diet treatment group, which was smaller than the other groups. The largest amount of astrocytes was found in the pre control group (K0). The result of normality test shows that the data of astrocytes amount is normal ($\alpha > 0.05$), so the next data is analyzed by parametric statistic test that is one-way ANOVA. One-way ANOVA test results obtained significant results that is 0.040 ($\alpha < 0.05$) which means there are significant differences between groups.

Table 2: Post Hoc anova test result.

GROUP	K0	K1	K2	K3
K0		0.266	0.030*	0.014*
K1	0.266		0.203	0.071

K2	0.030*	0.203		0.414
K3	0.14*	0.071	0.414	

*Significant difference

The result of Post-Hoc Anova test showed that there was a significant difference between the pre-control group (K0) and the 0.15 gram (K2) glucose treatment group and the 0.25 gram (K3) glucose treatment group. No significant difference was found between K1 with K0, K2, and K3. The most significant ratio of astrocytes is between K0 and K3.

4 DISCUSSION

High-calorie diets exaggeratedly cause metabolic abnormalities (Auer *et al.*, 2015). When the body gets a diet which is high in excessive calories for a long term, it can increase blood glucose levels. This can lead to conditions of hyperglycemia (Guyton & Hall, 2011). In addition, blood glucose levels, hyperglycemia also leads to adequate infusion of insulin (CDC, 2011). The condition of hyperglycemia can increase the formation of reactive oxygen species (ROS) in the body and can improve the health of brain tissue (Ding *et al.*, 2004).

The brain consumes 20% of oxygen and 25% of glucose present in the body. Aside of oxygen, glucose is very important for the brain cell, because different from other tissues, in normal conditions the brain can only use glucose as its source of energy (Farooqui, 2015). In conditions of hyperglycemia metabolic acidosis may occur in local brain tissue with increased of brain lactate (Kagansky *et al.*, 2001).

Astrocytes are neuroglia cells in the brain, composed of plastic cells that respond quickly to environmental changes in the brain (Kimelberg & Nedergaard, 2010). An important role of astrocytes in the central nervous system (CNS) is in the process of physiology and pathology occurring in the brain and body (Dong *et al.*, 2001; Biessels *et al.*, 1999). Cohen *et al.* (2016) reported that astrocyte from neonatal mice given low, medium, and high glucose exposure, responded to changes in environmental glucose levels with increased insulin, insulin receptors, and protein levels. Astrocytes secrete insulin but do not respond to stimuli like other insulin-producing cells. This has led to speculation that astrocytic insulin responds to glucose levels, and may be adaptive for cellular homeostasis rather than to affect the environment.

Astrocytes count between the pre-control group and the glucose diet treatment group of 0.15 gram were significantly different. This significant difference indicates a 0.15g glucose diet has an impact on astrocytes. This is due to the high levels of glucose in the blood followed by insulin insufficiency which results in reduced astrocytes as a protective response to neurons from environmental changes (Özdemir *et al.*, 2012; Kelleher *et al.*, 1993). Reduced astrocytes in the 0.15g glucose diet treatment group may also be due to high ROS that elevated blood glucose levels that cause astrocytic damage (Yang *et al.*, 2016; Takahashi *et al.*, 2012; Wang *et al.*, 2012).

High concentrations of glucose in the CNS environment affect astrocytes by increasing the ROS levels that cause oxidative stress (Hsieh *et al.*, 2013) as well as increased production of cytokine inflammation (Shin *et al.*, 2014). Under normal conditions, astrocytes have a major role in the CNS by maintaining extracellular homeostasis of neuroactive substances such as K +, H +, GABA, and glutamate. The more hyperpolarized membrane potentials compared with neurons can be found in astrocytes that provide the driving forces required for K + spatial buffering and glutamate transport (Kucheryavykh *et al.*, 2007, 2009; Olsen, 2012). When these functions are impaired it will affect brain physiology.

The amount of astrocytes between the pre-control group and the 0.25g glucose diet treatment group was significantly different (α =0.014). The difference was more significant than the amount of astrocytes between the pre-control group and the 0.15g glucose diet treatment group, indicating that astrocytes in the 0.25-gram diet were decreased more. Wang *et al.*, (2012) reported that in experimental animal astrocytes treated with exposure to high glucose levels (15 mM) did not induce apoptosis in astrocytes, while astrocyte experimental animals treated with exposure to extremely high glucose levels (30 mM) experienced apoptosis drastically. This may explain the higher the glucose diet is given, the more astrocytes that apoptosis leads to the less astrocytes that can be calculated.

The researcher found no significant difference for the amount of astrocyte between the pre control and post control groups. No significant difference also found in the astrocyte count between the post control group and the 0.15g glucose diet treatment group. The astrocyte count between the post control group and the 0.25g glucose diet treatment group also have no significant difference. And non-significant difference was founded in the astrocyte count between the 0.15g glucose diet treatment group and the 0.25g glucose diet treatment group.

The above non-significant differences were thought to be due to study limitations, differences in glucose levels administered between treatment groups were not much different and the majority of researchers looked at the impact of changes in astrocytic glucose levels by evaluation of morphological changes (Ogata & Kosaka, 2012; Wang *et al.*, 2012; Nardin *et al.*, 2007; Ding *et al.*, 2004; Auer *et al.*, 2015). While histological staining is best to be able to see astrocytes and its structure is silver impregnation, gold impregnation and Golgi impregnation (Lopez *et al.*, 2010).

5 CONCLUSION

Based on the study, it can be concluded that long-term high-calorie diets can cause a decrease in the amount of astrocyte in the cerebral cortex of mice and the increased calorie intake is associated with a decrease in the amount of astrocyte in the *cerebral cortex*.

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Non-Invasive Method on Slow-Twitch Quadriceps Muscle Fibers Dominate a High Level of Fitness

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Keywords: Fitness Level, Muscle Fiber Type, Non-Invasive Method, VO_2max.

Abstract: Previous studies have proved that there is a linear correlation between VO_2max and slow-twitch fibers in athletes. These studies were done using biopsy, the invasive method, to estimate the muscle fiber type. There is also a non-invasive method, which is convenient to use and not conflicted by ethical law in Indonesia. However, further study regarding muscle fiber shifting and its correlation with the level of fitness using a non-invasive method to estimate muscle fiber is still lacking. This research aims to determine the distribution and correlation between level of fitness and muscle fiber type in non-athletes estimated by a non-invasive method. Muscle fiber type in the quadriceps muscle group and level of fitness were determined in 33 untrained male students with an average age of 20.5 y.o. Muscle fiber type was determined by a non-invasive method by counting the maximal repetition with 80% 1RM weight. The level of fitness was determined a week later by the *Astrand ergocycle* method. The slow-twitch muscle fibers dominated a high fitness level ($p=0.002$). However, the correlation test between muscle fiber types and fitness level was not significant ($p=0.551$). This result showed that a high fitness level, which has a higher oxygen consumption, has numerous slow-twitch muscle fibers. It is rich in myoglobin delivering oxygen to maintain the aerobic activity. Yet there is no correlation between the level of fitness and muscle fiber type as estimated by a non-invasive method in quadriceps. Further studies are needed to analyze several factors that may contribute to it.

1 INTRODUCTION

Physical fitness has a direct relationship with the aerobic energy system, muscle ATP-PC capacities, and lactate metabolism (Robergs, 2003). Physical fitness can be determined by measuring VO_2max or maximal oxygen uptake (Ganong, 2010). VO_2max reflects the maximal amount of oxygen that has been consumed during intense physical activity.

Muscle fiber has been classified into slow-twitch (Type I) fiber and fast-twitch (Type II) fiber (Scott et al., 2001). The term mixed-twitch fiber has been used if there is an equal proportion of slow-twitch type and fast-twitch type in one muscle group. Slow-twitch fibers have a greater aerobic capacity, larger vascular and capillary, and are more resistant to fatigue than fast-twitch fibers (Ganong, 2010). Fast-twitch muscle was used for power and speed events due to its anaerobic capacity (Berning and Steen, 2005). Skeletal muscle consists of muscle tissues which can easily adapt due to the environment that change its constituent protein (Magaudda et al., 2004).

Several studies show that shifts in muscle fiber from fast-twitch fiber to slow-twitch fiber is possible due to long periods of endurance type events (Wilson et al., 2012). Athletes who are involved in long periods of endurance training have very high levels of VO_2max (Skinner, 2005) and a high percentage of slow-twitch fibers (Plowman, 2007). A previous study has proved that there is a linear correlation between VO_2max and slow-twitch fibers in athletes (Bergh et al., 1978). However, most of the research was carried out on athletes or trained men. Further study regarding muscle fiber shifting and its correlation with level of fitness in non-athletes and untrained men is still lacking. Moreover, a non-invasive method to identify muscle fiber type is still clearly unknown. The purpose of this study was to determine the distribution and

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correlation between level of fitness and muscle fiber type in non-athletes estimated by non-invasive method.

2 MATERIALS AND METHOD

Thirty-three untrained males aged 19–22 years participated in this study. Muscle fiber type in the *quadriceps* muscle group was determined by counting maximal repetition with 80% 1 RM weight during leg extension. This method is introduced and applied in muscle groups, not to an individual muscle (Kraps, 2001). Maximal repetition at a predetermined load and muscle fiber type have a fair to moderate relationship (Douris et al., 2006).

Level of fitness was determined using the *Astrand* method to measure predictive value of VO_2max while using the ergocycle. The *Astrand* method has been proven to be accurate in determining VO_2max in healthy young men and women (Hoehn et al., 2015). Then, the VO_2max was classified in low, average, high, and very high levels of fitness.

3 RESULTS

Frequency distribution of muscle fiber type of quadriceps muscle group among participants is presented at Fig. 1. Fifty-five percent participants have slow-twitch fibers, 39% mixed-twitch fibers, and 6% slow-twitch fibers. Frequency distribution of level of fitness among participants is presented at Fig. 2 where 21.2% participants have low level of fitness, 30.3% average, 33.3% high, and 15.2% very high.

The slow-twitch muscle fibers dominate low levels and high levels of fitness. There were five participants with slow-twitch and two participants with mixed-twitch muscle fibers at a low fitness level. In the high level of fitness, there were seven participants with slow-twitch and four participants with mixed-twitch muscle fibers. However, the significant difference between slow-twitch and mixed-twitch muscle fibers ($p=0.002$) was only in high level of fitness The participants with average level fitness were dominated by mixed-twitch muscle fibers, yet there was no significant difference. The fast-twitch muscle fiber type was detected in a small proportion ($p \geq 0.05$) on average and a very high level of fitness. The frequency of distribution based on classification of fitness level and muscle fiber type of participants is presented in Fig 3.

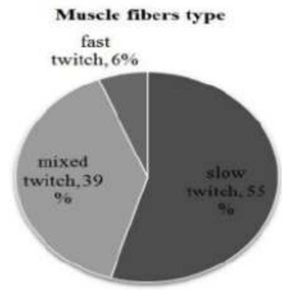

Figure 1: Frequency distribution of muscle fiber type of quadriceps muscle group (n=33).

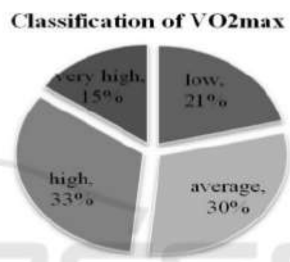

Figure 2: Frequency distribution of level of fitness (n=33).

P value was determined by the *Spearman* correlation test; $p = 0.551$ showed that there was no correlation between level of fitness and muscle fiber type (Table 1).

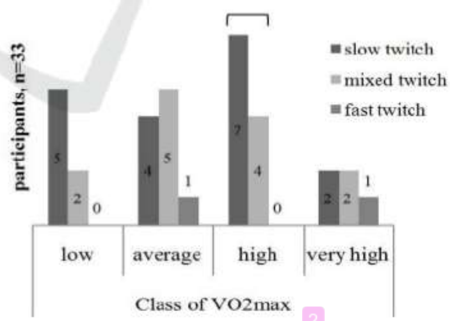

Figure 3: Frequency distribution of level of fitness on muscle fiber type.

*significant difference ($p<0.05$)) between slow and mixed-twitch muscle fibers.

Table 1. Correlation between level of fitness and muscle fiber type.

Spearman rho n=33	Classification of VO_2max	
Muscle fiber types	Correlation Coefficient	0.108
	Sig. (2-tailed)	0.551

4 DISCUSSION

The main finding in this study was that there is a significant difference between slow and mixed-twitch muscle fibers at a high level of fitness; however, there is no correlation between muscle fiber type and level of fitness in quadriceps of non-athletic young men. The domination of slow-twitch muscle fibers at a low and high fitness level is similar to the research by Barstow et al. (2000). Nine healthy participants were measured muscle fiber type recruitment based on the exercise intensity. The type I muscle (slow-twitch muscle fibers) was dominant in the low and/or high intensity of exercise (Barstow et al., 2000). Nevertheless, the correlation is contrary to the research from Barstow et al. (2000) and Bergh et al. (1978). One has to keep in mind that this study was done on untrained young men while their observation was done on athletes and trained men. Moreover, this study only uses *quadriceps* muscle to determine the muscle fiber type whereas the distribution between muscle fiber type varies in each muscle group.

There are some other factors that can influence level of fitness. Oxygen delivery is a primary factor in VO_2max limitation during exercises (Bassett and Howley, 2000). While athletes who have higher VO_2max also have a high percentage of slow-twitch fibers, VO_2max is not limited by the percentage of slow twitch muscle that someone is born with (Foss and Keteyian, 1998). Bergh's observation also found that VO_2max is higher in athletes than non-athletes in slow twitch fibers. This indicates that training and physical activities also influence VO_2max. Furthermore, studies regarding shift of muscle fiber from fast-twitch to slow twitch that was induced by training are still conflicted. It have already been cleared is muscle have the ability to alter its structural and functional properties to adapt to the environmental conditions imposed on it is known as muscle plasticity (Gransee et al., 2012). The mitogen-activated protein kinase signaling has been the possible pathway involved in exercise-induced adaptations in skeletal muscle (Hawley, 2002). Endurance exercise training can allegedly induce adaptive muscle fiber transformation and increase mitochondrial biogenesis (Wang et al., 2004). However, the endurance training procedure and period that can actually change the fast-twitch fiber to slow-twitch fiber are still being debated. The majority of evidence still suggests that cross-innervation is the only way to effectively change the fast-twitch fiber into a slow-twitch fiber (Foss and Keteyian, 1998).

5 CONCLUSION

In summary, a high level of fitness has slow-twitch muscle fibers type domination with the invasive method; however, the correlation between the level of fitness and muscle fiber type in quadriceps of young men has not been found in this study. Many other factors can influence level of fitness and muscle fiber type distribution. Factors and metabolic change pathways as well as the training procedure and period that can actually change the muscle fiber type from fast-twitch to slow-twitch are suggested as potential for further studies.

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Non-Invasive Method on Slow-Twitch Quadriceps Muscle Fibers Dominate a High Level of Fitness

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Keywords: Fitness Level, Muscle Fiber Type, Non-Invasive Method, VO_2max.

Abstract: Previous studies have proved that there is a linear correlation between VO_2max and slow-twitch fibers in athletes. These studies were done using biopsy, the invasive method, to estimate the muscle fiber type. There is also a non-invasive method, which is convenient to use and not conflicted by ethical law in Indonesia. However, further study regarding muscle fiber shifting and its correlation with the level of fitness using a non-invasive method to estimate muscle fiber is still lacking. This research aims to determine the distribution and correlation between level of fitness and muscle fiber type in non-athletes estimated by a non-invasive method. Muscle fiber type in the quadriceps muscle group and level of fitness were determined in 33 untrained male students with an average age of 20.5 y.o. Muscle fiber type was determined by a non-invasive method by counting the maximal repetition with 80% 1RM weight. The level of fitness was determined a week later by the *Astrand ergocycle* method. The slow-twitch muscle fibers dominated a high fitness level ($p=0.002$). However, the correlation test between muscle fiber types and fitness level was not significant ($p=0.551$). This result showed that a high fitness level, which has a higher oxygen consumption, has numerous slow-twitch muscle fibers. It is rich in myoglobin delivering oxygen to maintain the aerobic activity. Yet there is no correlation between the level of fitness and muscle fiber type as estimated by a non-invasive method in quadriceps. Further studies are needed to analyze several factors that may contribute to it.

1 INTRODUCTION

Physical fitness has a direct relationship with the aerobic energy system, muscle ATP-PC capacities, and lactate metabolism (Robergs, 2003). Physical fitness can be determined by measuring VO_2max or maximal oxygen uptake (Ganong, 2010). VO_2max reflects the maximal amount of oxygen that has been consumed during intense physical activity.

Muscle fiber has been classified into slow-twitch (Type I) fiber and fast-twitch (Type II) fiber (Scott et al., 2001). The term mixed-twitch fiber has been used if there is an equal proportion of slow-twitch type and fast-twitch type in one muscle group. Slow-twitch fibers have a greater aerobic capacity, larger vascular and capillary, and are more resistant to fatigue than fast-twitch fibers (Ganong, 2010). Fast-twitch muscle was used for power and speed events due to its anaerobic capacity (Berning and Steen, 2005). Skeletal muscle consists of muscle tissues which can easily adapt due to the environment that change its constituent protein (Magaudda et al., 2004).

Several studies show that shifts in muscle fiber from fast-twitch fiber to slow-twitch fiber is possible due to long periods of endurance type events (Wilson et al., 2012). Athletes who are involved in long periods of endurance training have very high levels of VO_2max (Skinner, 2005) and a high percentage of slow-twitch fibers (Plowman, 2007). A previous study has proved that there is a linear correlation between VO_2max and slow-twitch fibers in athletes (Bergh et al., 1978). However, most of the research was carried out on athletes or trained men. Further study regarding muscle fiber shifting and its correlation with level of fitness in non-athletes and untrained men is still lacking. Moreover, a non-invasive method to identify muscle fiber type is still clearly unknown. The purpose of this study was to determine the distribution and

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correlation between level of fitness and muscle fiber type in non-athletes estimated by non-invasive method.

2 MATERIALS AND METHOD

Thirty-three untrained males aged 19–22 years participated in this study. Muscle fiber type in the *quadriceps* muscle group was determined by counting maximal repetition with 80% 1 RM weight during leg extension. This method is introduced and applied in muscle groups, not to an individual muscle (Kraps, 2001). Maximal repetition at a predetermined load and muscle fiber type have a fair to moderate relationship (Douris et al., 2006).

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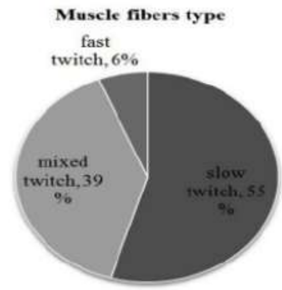
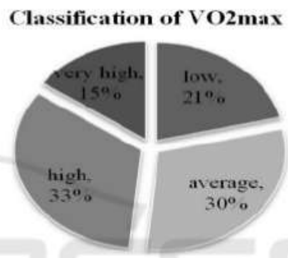


Figure 1: Frequency distribution of muscle fiber type of quadriceps muscle group (n=33).



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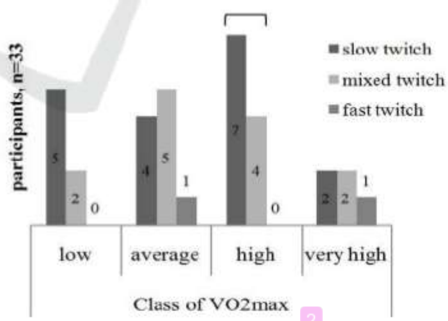

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