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Dose-Dependent Effects of Fluvastatin on Locomotion, Muscle Function, and Sleep Patterns in Drosophila Melanogaster

Suvarthy Dey[1], Tathagata Roy Chowdhury[2*]

[1]MBBS, MPH (Occupation and environmental health; major: Occupational health and safety); Msc in biomedicine, India

[2]Assistant Professor, Department of Computer Science and Engineering, Techno Engineering College Banipur, India

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[*]**Corresponding author:** Tathagata Roy Chowdhury, Assistant Professor, Department of Computer Science and Engineering, Techno Engineering, College Banipur, India

ABSTRACT

Fluvastatin is a drug of the statin group which have been widely used for treatment of hypercholesterolemia. Fluvastatin has shown to induce lower locomotion and general activity in flies. The dose response curve however has not been established yet. Accordingly, we aimed to examine different concentrations of Fluvastatin in flies to establish the dose response curve. This is very important to obtain the dose-curve response. Here we tried to identify dose response curve by using fruit fly (Drosophila melanogaster) activity monitoring system (DAMS). We have used CSORC variant of virgin males fruit fly and divided in three groups among which one group was control group and two different concentrations of Fluvastatin treated other two groups. To identify differentiation between two different concentrations of this experiment was done by using DAMS since it is not clearly understood at which concentration the Fluvastatin has the highest effect, i.e., make the activity of the flies lower. We have done DAMS for 5 hours. 5hours later we have done DAMS analysis and found that, the control group flies were more active than the flies' treated with Fluvastatin. The result does not show any difference in effect between the two concentrations. At the same time, we also got contradictory results regarding sleep duration. The control group flies had lower sleep duration than the other two groups of flies which Fluvastatin treated. This finding is completely opposite than the findings of another research

Keywords: Fluvastatin; Drosophila melanogaster; Statin-induced myopathy; Dose-response curve; Locomotion; Sleep duration; HMG-CoA reductase; Muscle activity; Drosophila Activity Monitoring System (DAMS)[Cholesterol

INTRODUCTION

The 3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMGCR) Mevalonate (MVA) pathway is involved in the production of sterols, especially cholesterol, which are essential for the formation of cell membrane, fatty acids, and steroid hormones. Cholesterol plays a crucial role in many of the biological processes of human and fly. In addition, the MVA pathway is also known to produce other steroids, prenylated proteins and isoprenoids. Notably, HMGCR is the rate limiting enzyme in the MVA pathway which is a precursor of juvenile hormone family components [1].

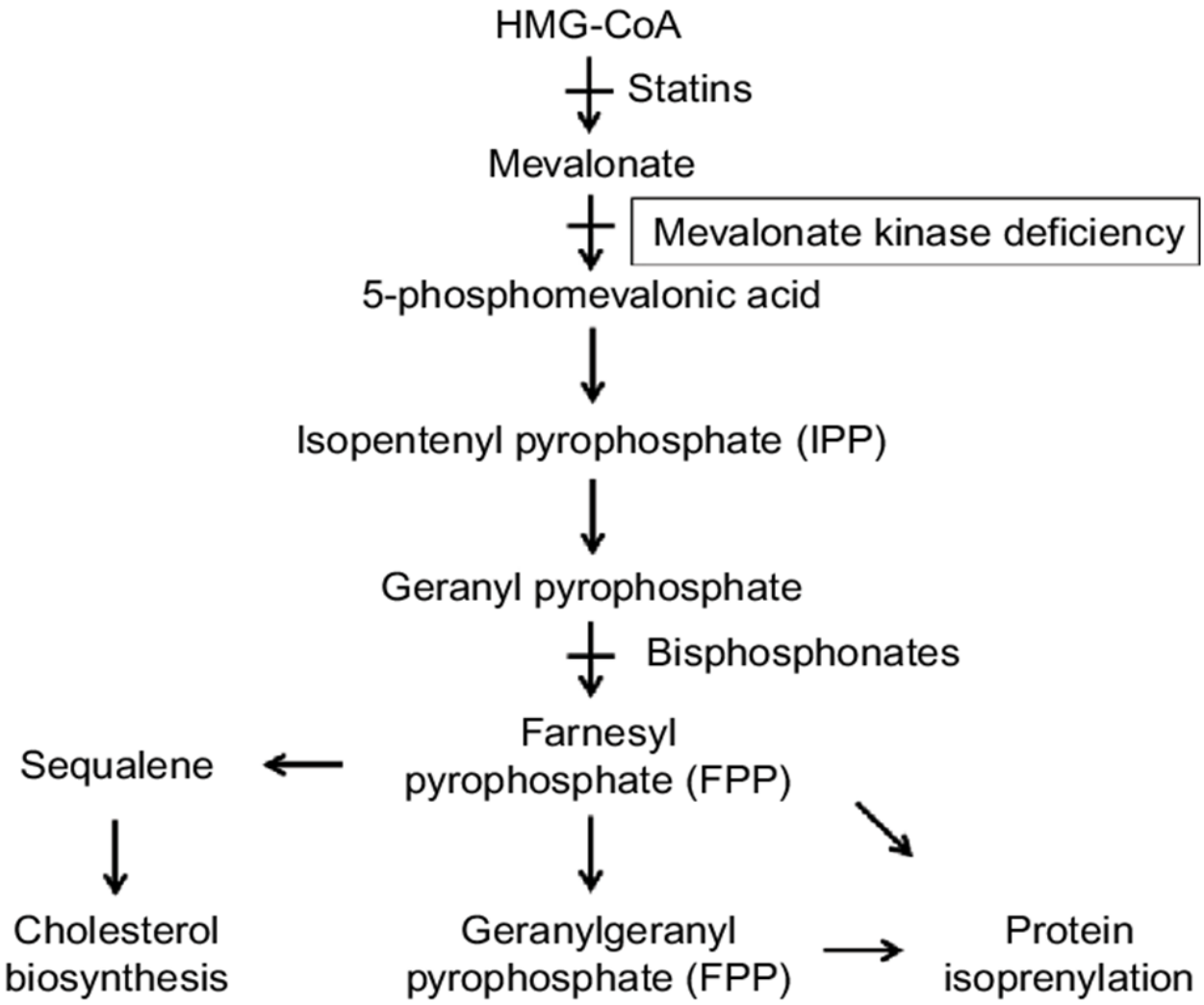

Figure 1. Mevalonate pathway.

In the mevalonate pathway HMG-CoA is converted into mevalonate by the action of HMG-CoA reductase (HMGCR). Then by the action of mevalonate kinase enzyme, mevalonate convert into 5-phosphomevalonic acid which ultimately convert into geranyl pyrophosphate and later produces cholesterol, geranylgeranyl pyrophosphate (FPP) and protein isoprenylation. In this point the statins inhibit the action of mevalonate kinase and prevent

conversion of mevalonate into5-phosphomevalonic acid which ultimately preventing the production of end products including cholesterol, geranylgeranyl pyrophosphate (FPP) and protein isoprenylation [2].

Since the MVA pathway affects various metabolic pathways, HMGCR inhibitors, also known as statins, have been widely used clinically as an effective treatment for hypercholesterolemia. It is safe, well tolerated and very effective drug in the treatment of hypercholesterolemia, which is one of the risk factors for atherosclerosis. As a result, it is a frequently prescribed medicine [3].However, statins are associated with many side effects, but the most severe effect is mainly myopathy, myalgia, or rhabdomyolysis, depending on the exposure duration. It is known that the statins block the formation of HMGCR which leads to statin induced myopathy [4].The mechanism through which statin induced myopathy occurs is still poorly understood. Previous studies show that feeding fruit fly (Drosophila melanogaster) with Fluvastatin reduced locomotion and induced myopathy [5].

Generally, statins are prescribed in tablet form to the patient which are lactones. Lactones are three times more potent to induce myopathy. Later these lactones affect the mitochondria and slow down the cholesterol production within the liver. The pathogenesis of developing statin-associated myotoxicity (SAM) is still not clear. Many researchers have proposed many possible mechanisms and frameworks in SAM. In a previous study, researchers used myoblast cell model to investigate the mechanism of statin induced myotoxicity. From this previous study it has come to know that, after administration, statin remains as acid and lactone form within the body [6]. The acid form turns into lactone, but the lactone does not have any therapeutic effect. In the myoblast cell model, the lactone showed three times more potent and interrupt with the mitochondrial oxidative phosphorylation pathway that produces cell energy ATP. Lactone statin significantly inhibit the CI-, CII-, CIII along Qo and Qi sites of complex III of the mitochondria respiratory chain, glycerol-3-phosphate dehydrogenase (G3PDH) and decrease the basal oxygen consumption which ultimately leads to reduction of ATP production without altering mitochondrial membrane potential. [6] Another group of researchers has proposed a general framework of interaction between wide spectrum of anti-HMGCR induced sustained autoimmune muscle injury and inadequate response to regeneration. In this study the researchers used HMGCR protein as autoantigen and anti-HMGCR as autoantibodies targeting HMGCR protein [7].

Another researcher group proposed a general framework of interaction between host immunogenic background with environmental triggers along with autoimmune muscle injury and has predicted a schematic of postulated patho-mechanism of statin induce myopathy [8]. They did this in a meta-analysis based on data of several studies in recent years which provided clues of immune pathogenesis of statin induced anti-HMGCR myopathy. According to their experiment, statin exposure may be causing overexpression of HMGCR or mimicking HMGCR in multiple tissues specially the cryptic epitopes variant of HMGCR which become unmasked by statins drug. Later they are processed by antigen presenting cells under various situation and recognize over expressed HMGCR receptor as antigen. This phenomenon stimulates HLA molecule and increasing T-cell activation and initiate autoimmune response by producing anti-HMGCR antibody. The process of myofiber repair cannot alter the ultimate outcome of muscle atrophy and degeneration of myofibers [8].

Moreover, in another study, another mechanism of statin induced muscle injury was proposed. According to this study, depending on the genetic factors' statin induced effects on skeletal muscles includes, HMGC-CoA reductase pathway mediated effects along with cellular and sub-cellular effects. According to this proposed mechanism anti-HMGCR activity of statin can alter the muscle cell membrane stability, along with protein signaling and activity and reduce cell membrane cholesterol and impair mitochondrial functions. In addition, along with reduction of cell membrane cholesterol HMGC-CoA reductase inhibition is also affecting the ion channel activity leading to myocyte damage and resulting myopathy [9].

A phase IV clinical trial among people who have taken Fluvastatin included mostly of the female participants (around 85.7%) from the age 40-49years. Only 14.29% of the participants was male of the same age as the female participants. Most of the participants had coronary heart disease along with other systemic conditions such as, hypothyroidism, type 2 diabetics, osteoporosis etc. According to this report, 75% of the participants developed movement disorders followed by developing paraesthesia, muscle spasm, muscle weakness, joint stiffness, Raynaud's phenomenon etc. within less than 1month after taking Fluvastatin. Among the respondents around 68.42% patients developed low back pain. But it is still not proven that Fluvastatin is affecting locomotion system. Depending on the other clinical history of participants these may be a reason of drug-drug interaction or may be a reason of statin induce myopathy. So, it needs to do proper evaluation [10].

Regarding statin effect on sleep there are also some controversies. In 2015, a meta-analysis was published which said statin is affecting sleep duration, especially at night. Those people who are using statin reported that their night sleeping was unrestful, and they felt drowsy at the daytime and sometimes they had to take a nap in the daytime [11].

Later, in 2014, a study found that, after using multiple statistical methodologies and databases it was strongly suggested that statin is causing sleep disturbance including insomnia [12].

But in 2015, a group of researchers did a systemic review on sleep changes following statin use and they did not find any significant adverse effect on sleep duration in any level of sleep cycle. According to this research paper statin significantly reduces wake time and affects the sleep cycle [13].

Lastly, in 2016 another research paper was published saying that, statin is improving cardiovascular disease but at the same time causing sleep deprivation. According to thi study, statin improved the overall cardiac function along with improvement in inflammation, oxidative stress, and highly sensitive C-reactive protein (hs-CRP) to the group who were treated with statin than the control group. But the mechanism and other relative factors still not confirmed because the study was done on healthy people with 48 hours sleep deprivation [14].

MATERIALS AND METHODS

Drosophila melanogaster:

Fruit fly (Drosophila melanogaster) which has been a particularly valuable model system to study genetics, development, physiology, and behavior for. Over 100years 70% of the genes involved in human diseases have a homologue in fruit fly which facilitates their study in this model that have several advantages such as low cost, easy manipulation, rapid growth, and reproduction among many others [15].

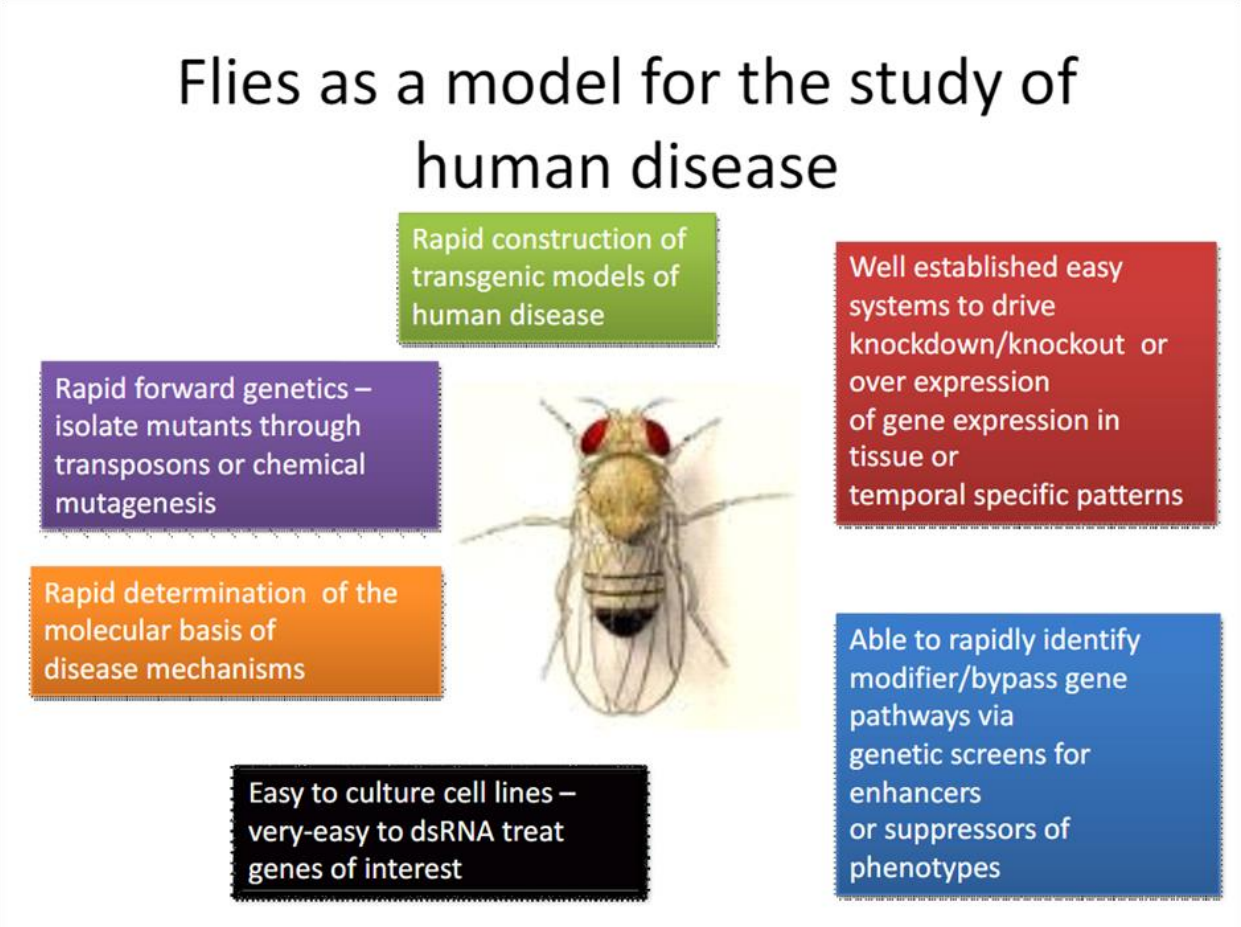

Figure 2. Advantage of using Drosophila melanogaster as a model

In this experiment we used CSORC variant of male Fruit flies. These were chosen because they grow very fast and have shorter reproductive and life cycle. Moreover, one of our bachelor students have made a Cl^- channel knockdown on this variant which is not published yet. According to that student study Fluvastatin is affecting the Cl^- channel and decreasing total activity and movement among the CSORC variant of male fruit flies (Bloomington Drosophila stock center).

Drosophila Activity Monitor System (DAMS):

The Drosophila Activity Monitor system (DAMS) is a widely used device which record fruit fly behavior. The DAMS monitor has 32chambers placed in a temperature, humidity and light controlled incubator which allowing control environmental response, physiological use of temperature, light and darkness response and circadian rhythms. It allows monitoring over many days to weeks. Each chamber contains two open ended glass tubes. During an experiment, one end of the glass tube contains fly food which is closed by a rubber cap and the other end contains cotton cap. An infrared beam passes through the glass tubes while flies walking within the glass tube [16].

Experimental procedures:

Here, we have done an experiment to observe the effect of Fluvastatin on movement, total activity, and sleep duration of CSORC variant of fruit flies. For this we collected 45 CSORC male virgin flies divided into 3 groups: 15 flies for control group, 15 flies for the group that was fed with food mixed with 0.5mM of Fluvastatin and 15 flies for the group who were feed with food mixed with 1 mM Fluvastatin. We observed the flies' movement, total activity, and sleep duration for 5 hours in Drosophila Activity Monitor System (DAMS).

We prepared 0.5 mM and 1 mM concentrations of Fluvastatin from a 5 mM stock solution (Sigma Aldrich). Three petri dishes containing 12 ml of fly food was prepared following fisher thermo scientific recipe, also known as jazz mix.

Table: Preparation procedure of food with two different concentrations of Fluvastatin

Control Group flies (n=15)	Only food. The food volume was 12ml.
For 0.5mM flies' group (n=15)	1.2ml of 5mM Fluvastatin mixed with10.8ml of boiling food = 12ml
For 1mM flies' group (n=15)	2.4ml of 5mM Fluvastatin mixed with9.6ml of boiling food = 12ml

We collected 45 male virgin flies in one day. Food was placed in one end of a glass tube and the food containing side was closed with a rubber cap. Each glass tube contained a single fly. The other end of the glass tube was closed with a cotton pad to trap the fly inside glass tube and to ensure proper oxygenation. Then the class tubes were put within the DAMS chamber inside the incubator at 25° C temperature for 5hours.

RESULTS

Per Hour Movement Activity:

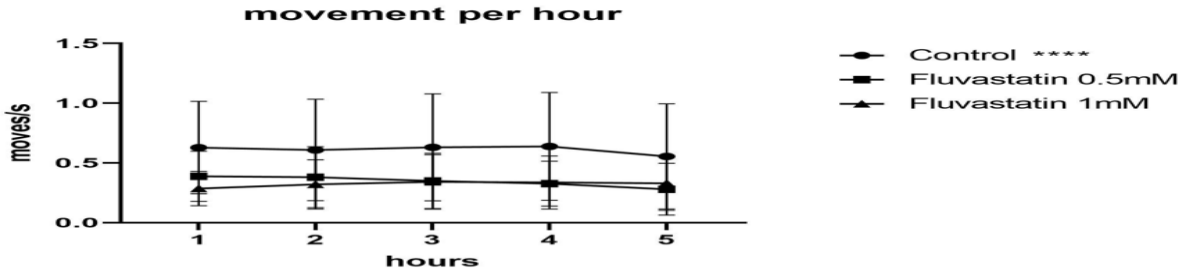

Figure 3: Per hour Movement activities of flies after feeding two different concentrations of Fluvastatin.

When observing per hour movement over 5 hours, we found that the control group were more active than the two Fluvastatin groups over all time-points **(Figure 3)**. In between two statin groups the flies who had a diet containing 0.5mM Fluvastatin showed a little more activity than the group who had a diet containing 1mM Fluvastatin **(Figure 3)**. At 3 hours, both groups of flies that had Fluvastatin in their diet showed similar level of activity, but there is no significant difference between the two Fluvastatin experimental groups **(Figure 3)**.

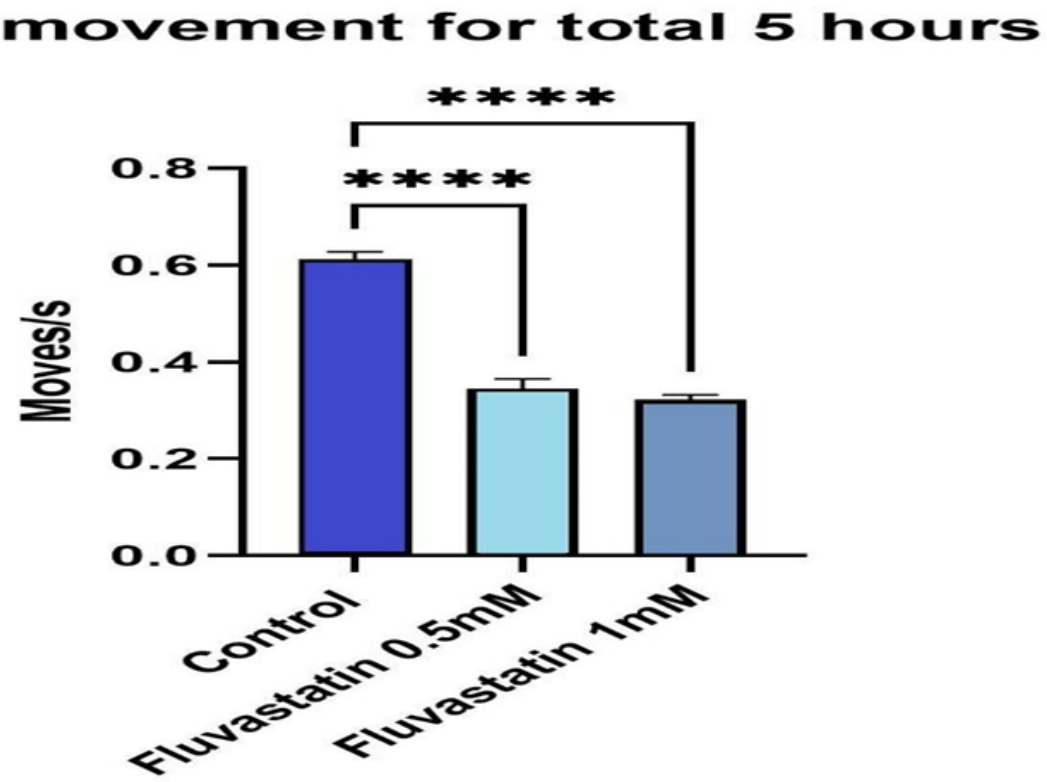

Figure 4: Total Movement activities of flies for total 5hours after feeding two different concentrations of Fluvastatin.

When investigating total activity over 5 hours movement, we have found that, the control group were more active than the two Fluvastatin groups **(Figure 4)**. The flies who were fed a diet containing 0.5mM Fluvastatin showed a little more activity than the group who was fed a diet containing 1mM Fluvastatin **(Figure 4)**. But there is no significant difference between the two different Fluvastatin experimental groups **(Figure 4)**.

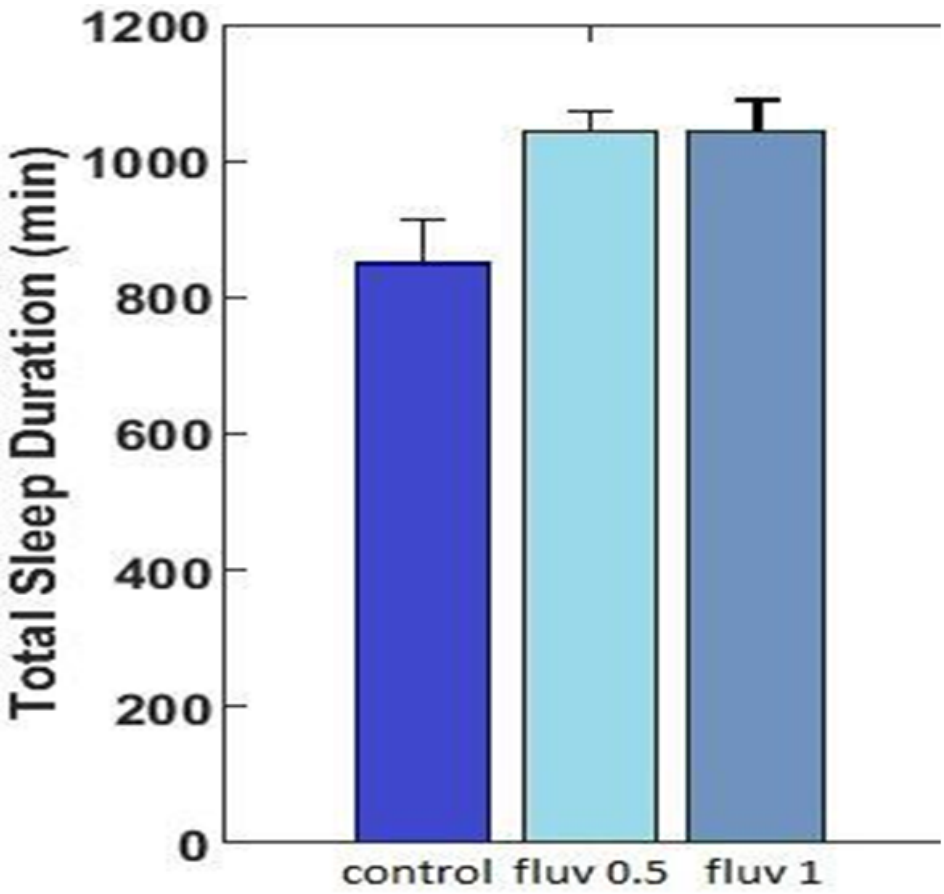

Figure 5: Total sleep duration of flies after feeding with Fluvastatin.

When investigating sleep duration, we found completely different result compering other related studies. The control group had shorter sleep duration rather than the two different Fluvastatin groups **(Figure 5)**. The Fluvastatin groups had the same sleep duration, and this was longer than the control group **(Figure 5)**. But the difference between the Fluvastatin groups and the control group was not significant.

DISCUSSION

To understand the effect of Fluvastatin on movement, locomotion and sleep we took the CSORC variant of fruit flies and divided them into a control group and two groups fed with different concentrations of Fluvastatin (0.5mM and 1mM of Fluvastatin respectively). We used two different concentrations of Fluvastatin to identify the dose at which we can see an effect. We monitored the total event by DAMS for 5 hours Control group flies showed more activity than the groups treated with Fluvastatin which agrees with other findings [10]. This decrease in the activity of the flies occurred from the lower concentration of Fluvastatin tested. To identify Fluvastatin's dose response relationship, a longer duration experiment is needed. Moreover, the actual mechanism of how Fluvastatin is affecting the total musculoskeletal system is still not clear.

At the same time, in the case of sleep duration, the two concentrations of Fluvastatin showed longer sleep duration than the control group. Previously, in different experiments, it has been shown that statins lower sleep duration. However, in our experiment, we found that the control group had shorter sleep duration than the two Fluvastatin groups. Based on our findings we can interpret Fluvastatin is increasing sleep duration which is opposite with the recently published research paper [17]. According to this paper, HMGCR plays a very important role in sleep modulation. Whenever Fluvastatin is blocking the HMGCR it is also affecting the pan neuronal HMGCR expression which in turn is altering the fly sleep behavior. Loss of HMGCR expression in different parts of pars intercereblis (PI; is like mammalian hypothalamus) is affecting the corticotropin releasing factor homologue expressing neurons which increasing the sleep latency, at the same time, decrease sleep duration [17]. But the reason being controversial result of our experiment has not excluded yet.

A student from our lab, Petricia Daller has done the same experiment as described here on W118 wild type variant of fruit flies (result is still unpublished). According to this experiment Fluvastatin increased the sleep duration on the flies but reduced movement and total activity. In that experiment, the student knocked-down the expression of ClC-1 in the skeletal muscle in W118 wild type of fruit flies. ClC-1 is a Cl- channel in mammalian skeletal muscle which plays an important role in muscle contraction by membrane repolarization. Reduction of ClC-α causes muscle hyperexcitability [18]. According to our student's experiment, the flies who showed over expression of ClC-1 in the skeletal muscle were more active. So, based on that experiment we can say over expression of ClC-1 might be reverse the statin induced movement problem which ultimately might be affecting the locomotor system. So, it is very important to clear out this controversy and need more experiment.

CONCLUSION

Based on the different research articles and available data it is confirmed that statin affecting the movement and activity of fruit flies, but the pathogenesis is still not clear. The effect on sleep is also not clear. At this point it is necessary to be clear about the pathogenesis of reduced muscle activity and in which point the Fluvastatin is affecting the movement and total activity. More experiments are needed on whether statin alone affects locomotion system or if it is a result of drug-drug interaction. There is also controversy about the effect of statin on sleep. Some reports showed statin has effect on the length of sleep duration but at the same time some reports showed statin causing insomnia. In our lab experiment we have observed an increase in sleep duration following statin use. More studies on this topic are therefore needed.

REFERENCES

1. IrinaBuhaescu, HassaneIzzedine. Mevalonate pathway: A review of clinical and therapeutical implications. Clin Biochem: 2007.
2. Grant S Schulert. Mevalonate kinase deficiency: Current perspectives. July 2016. The Application of Clinical Genetics 2016;9(1):101-110.

3. Alice Brockington, Paul R Heath, Hazel Holden, Paul Kasher, Florian LP Bender, et al. Downregulation of genes with a function in axon outgrowth and synapse formation in motor neurones of the VEGF$^{\delta/\delta}$ mouse model of amyotrophic lateral sclerosis. BMC Genomics 11:203. 2010.
4. Yesser Hadj Belgacem and Jean-René Martin. Neuroendocrine control of a sexually dimorphic behavior by a few neurons of the pars intercerebralis in Drosophila. 2002; 99(23) 15154-15158.
5. Panel Tom JJ, Schirris G. et al. Statin-Induced Myopathy Is Associated with Mitochondrial Complex III Inhibition; Cell Metabolism; 2015;399-407.
6. Mohassel Payam, Mammen Andrew L. Anti-HMGCR Myopathy; Journal: Journal of Neuromuscular Diseases 5(1):11-20:2018.
7. Mohassel, Payam, Mammen, Andrew L. Anti-HMGCR Myopathy; Journal of Neuromuscular Diseases, 5(1)11-20:2018.
8. Natalie C Ward, Gerald F, Watts Robert H Eckel. Statin Toxicity. Mechanistic Insights and Clinical Implications. 2019.
9. Marjel van Dam, John Kastelein, on behalf of the Fluvastatin Collaboration Group, Department of Vascular Medicine, Academic Medical Centre, Amsterdam, The Netherlands. Efficacy and Tolerability of Fluvastatin in a Titration Dosage Regimen in Hyperlipidaemic Patients: Results of a Multicentre Phase IV Study. Clinical Drug Investigation: 2001;21(7).
10. Marlena Broncel, Paulina Gorzelak-Pabiś, et al. Lipid and Blood Pressure Meta-analysis Collaboration (LBPMC) Group. Sleep changes following statin therapy: a systematic review and meta-analysis of randomized placebo-controlled polysomnographic trials. Arch Med Sci. 2015; 11(5): 915–926.
11. Mitsutaka Takada, Mai Fujimoto, Kohei Yamazaki, Masashi Takamoto, Kouichi Hosomi; Association of Statin Use with Sleep Disturbances: Data Mining of a Spontaneous Reporting Database and a Prescription. Published: International Journal of Medical Sciences; 2016.
12. Marlena Broncel , Paulina Gorzelak-Pabiś , Amirhossein Sahebkar , Katarzyna Serejko , Sorin Ursoniu , et al. Lipid and Blood Pressure Meta-analysis Collaboration (LBPMC) Group. Sleep changes following statin therapy: a systematic review and meta-analysis of randomized placebo-controlled polysomnographic trials; 2015;11(5):915-926.
13. Wei Ren Chen, Hong Bin Liu , Yuan Sha , Yang Shi , Hao Wang , Da Wei Yin , et al. Effects of Statin on Arrhythmia and Heart Rate Variability in Healthy Persons With 48-Hour Sleep Deprivation; J Am Heart Association 2016;5(11):e003833.
14. Thomas R Jahn, Elke Malzer, John Roote, Anastasia Vishnivetskaya, Sara Imarisio, et al. Modeling Serpin Conformational Diseases in Drosophila melanogaster; Elsevier: 499.
15. Drosophilia Activity Monitoring System-Trikinetic DAMSystem Overview.
16. Ahmed M Alseli, Sifang Liao, Mohammed H. Al-Sabri, Lukas Vasionis, et al. The Statin Target HMG-Coenzyme a Reductase (Hmgcr) Regulates Sleep Homeostasis in Drosophila. Pharmaceuticals 2022;15(1): 79.

17. Edoardo C Aromataris Grigori Y Rychkov. ClC-1 chloride channel: Matching its properties to a role in skeletal muscle. Clin Exp Pharmacol Physiol. 2006;33(11):1118-1123.