2. Perlu dilakukan penelitian lanjutan mengenai toksisitas statin yang menyebabkan disfungsi mitokondria akibat beragam obat statin pada berbagai tipe jaringan otot skeletal dan otot jantung.
3. Perlu dilakukan penelitian lebih lanjut mengenai keterlibatan proses stress oksidatif dan apoptosis pada disfungsi mitokondria akibat pemberian statin
4. Perlu dilakukan penelitian dengan durasi perlakuan lebih lama, untuk menilai lebih lanjut terkait miotoksistas akibat statin

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LAMPIRAN

Lampiran 1 .Rekomendasi Persetujuan Etik

KEMENTERIAN PENDIDIKAN DAN KEBUDAYAAN
UNIVERSITAS HASANUDDIN FAKULTAS KEDOKTERAN
KOMITE ETIK PENELITIAN KESEHATAN
RSPTN UNIVERSITAS HASANUDDIN
RSUP Dr. WAHIDIN SUDIROHUSODO MAKASSAR
Sekretariat : Lantai 2 Gedung Laboratorium Terpadu
JL.PERINTIS KEMERDEKAAN KAMPUS TAMALANREA KM.10 MAKASSAR 90245.
Contact Person: dr. Agussalim Bukhari.,MMed,PhD, SpGK TELP. 081241850858. 0411 5780103, Fax : 0411-581431

REKOMENDASI PERSETUJUAN ETIK

Nomor : 560/UN4.6.4.5.31/ PP36/ 2020

Tanggal: 17 September 2020

Dengan ini Menyatakan bahwa Protokol dan Dokumen yang Berhubungan Dengan Protokol berikut ini telah mendapatkan Persetujuan Etik :

No Protokol	UH20090473	No Sponsor Protokol	
Peneliti Utama	**dr. Zulfahmidah**	Sponsor	
Judul Peneliti	Efek Pemberian Simvastatin Terhadap Kadar Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-Alpha (PGC-1a) Otot Skeletal Dan Otot Jantung Tikus Wistar (Rattus Norvegicus)		
No Versi Protokol	**1**	Tanggal Versi	**9 September 2020**
No Versi PSP		Tanggal Versi	
Tempat Penelitian	**Laboratorium Biofarmaka Fakultas Farmasi Universitas Hasanuddin Makassar**		
Jenis Review	☐ Exempted ☒ Expedited ☐ Fullboard Tanggal	Masa Berlaku **17 September 2020** sampai **17 September 2021**	Frekuensi review lanjutan
Ketua Komisi Etik Penelitian Kesehatan FKUH	Nama **Prof.Dr.dr. Suryani As'ad, M.Sc.,Sp.GK (K)**	Tanda tangan	
Sekretaris Komisi Etik Penelitian Kesehatan FKUH	Nama **dr. Agussalim Bukhari, M.Med.,Ph.D.,Sp.GK (K)**	Tanda tangan	

Kewajiban Peneliti Utama:

- **Menyerahkan Amandemen Protokol untuk persetujuan sebelum di implementasikan**
- **Menyerahkan Laporan SAE ke Komisi Etik dalam 24 Jam dan dilengkapi dalam 7 hari dan Lapor SUSAR dalam 72 Jam setelah Peneliti Utama menerima laporan**
- **Menyerahkan Laporan Kemajuan (progress report) setiap 6 bulan untuk penelitian resiko tinggi dan setiap setahun untuk penelitian resiko rendah**
- **Menyerahkan laporan akhir setelah Penelitian berakhir**
- **Melaporkan penyimpangan dari prokol yang disetujui (protocol deviation / violation)**
- **Mematuhi semua peraturan yang ditentukan**

Lampiran 2. Surat Keterangan Selesai Penelitian

RUMAH SAKIT UNHAS	**SURAT KETERANGAN SEMENTARA SELESAI PENGAMBILAN DATA/ANALISA BAHAN HAYATI**
	Diterbitkan oleh **Laboratorium Penelitian**
FORMULIR 3 BIDANG PENELITIAN DAN INOVASI	Ditujukan kepada **KEPALA BIDANG PENELITIAN DAN INOVASI**

Dengan hormat,

Dengan ini menerangkan bahwa peneliti/ mahasiswa berikut ini:

Nama : dr. Zulfahmidah

NIM / NIP : P062191021

Institusi : Ilmu Biomedik/ Biokimia dan Biologi Molekuler, Sekolah Pasca Sarjana, Universitas Hasanuddin Makassar

Kode penelitian : 200923_1

TELAH SELESAI melakukan pengambilan data/ analisa bahan hayati

Pada tanggal : 02 Oktober 2020

Jumlah Subjek : 48 responden/sampel

Jenis Data : Hasil ELISA

Dengan nama pendamping/ pembimbing

Staff : Sulhidayah

Konsultan :

Surat keterangan ini juga merupakan penjelasan bahwa peneliti/mahasiswa di atas tidak mempunyai sangkutan lagi pada unit/ instalasi kami

Kepala Ruang

Sulhidayah

NIP.

Catatan:

1. *lembaran ini agar diisi dan diberikan kepada mahasiswa/peneliti untuk diserahkan kepada Bidang Penelitian dan Inovasi setelah pengambilan data / analisa bahan hayati selesai*
2. *Surat pengantar ini berlaku 2 x 24 jam hari kerja di unit penelitian RSUH*

Lampiran 3. Dokumentasi Penelitian

Kondisi kandang hewan coba

Pengukuran berat badan hewan coba dengan menggunakan timbangan neraca digital. Terlihat bahwa seluruh badan tikus dipastikan menapak pada dasar wadah untuk memastikan hasil pengukuran yang valid.

Pengukuran panjang badan dan lingkarperut tikus hewan coba

Pengambilan jaringan otot skeletal dan otot jantung tikus setelah diterminasi

Pengambilan jaringan otot skeletal tikus (otot gastrocnemius)

Pembuatan larutan standar untuk metode ELISA

Pemberian solution substrat pada tiap well dengan menggunakan micropipet

Inkubasi Well yang telah diberikan solution substrat

Lampiran 4. Analisis Data Statistik SPSS

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
PGCjantung	1	8	3.9985959	.43079792	.15231007	3.6384398	4.3587520	3.25136	4.64357
	2	8	3.8555073	.26061925	.09214282	3.6376242	4.0733905	3.57496	4.42937
	3	8	4.2074104	.44792879	.15836674	3.8329326	4.5818883	3.43232	4.73007
	Total	24	4.0205045	.40000811	.08165131	3.8515959	4.1894131	3.25136	4.73007
PGCGastrocnemius	1	8	4.8918384	.51093590	.18064312	4.4646853	5.3189915	3.87203	5.40976
	2	8	4.2561778	.51185236	.18096714	3.8282585	4.6840971	3.72456	5.27224
	3	8	4.0122108	.42366675	.14978881	3.6580165	4.3664051	3.48315	4.81104
	Total	24	4.3867423	.59772527	.12201016	4.1343451	4.6391396	3.48315	5.40976

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
PGCjantung	1.687	2	21	.209
PGCGastrocnemius	.140	2	21	.870

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PGCjantung	Between Groups	.501	2	.251	1.655	.215
	Within Groups	3.179	21	.151		
	Total	3.680	23			
PGCGastrocnemius	Between Groups	3.300	2	1.650	7.045	.005
	Within Groups	4.918	21	.234		
	Total	8.217	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable		(I) Perlakuan	(J) Perlakuan	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
PGCjantung	Tukey HSD	1	2	.14308858	.19453994	.745	-.3472630	.6334402
			3	-.20881454	.19453994	.541	-.6991661	.2815371
		2	1	-.14308858	.19453994	.745	-.6334402	.3472630
			3	-.35190312	.19453994	.191	-.8422547	.1384485
		3	1	.20881454	.19453994	.541	-.2815371	.6991661
			2	.35190312	.19453994	.191	-.1384485	.8422547
	Games-Howell	1	2	.14308858	.17801308	.708	-.3345123	.6206894
			3	-.20881454	.21972342	.619	-.7839922	.3663631
		2	1	-.14308858	.17801308	.708	-.6206894	.3345123
			3	-.35190312	.18322206	.178	-.8451230	.1413167
		3	1	.20881454	.21972342	.619	-.3663631	.7839922
			2	.35190312	.18322206	.178	-.1413167	.8451230
PGCGastrocnemius	Tukey HSD	1	2	.63566053*	.24196106	.040	.0257807	1.2455404
			3	.87962756*	.24196106	.004	.2697477	1.4895074
		2	1	-.6356605*	.24196106	.040	-1.2455404	-.0257807
			3	.24396704	.24196106	.580	-.3659128	.8538469
		3	1	-.8796276*	.24196106	.004	-1.4895074	-.2697477
			2	-.24396704	.24196106	.580	-.8538469	.3659128
	Games-Howell	1	2	.63566053	.25569717	.064	-.0335709	1.3048920
			3	.87962756*	.23466705	.006	.2630260	1.4962291
		2	1	-.63566053	.25569717	.064	-1.3048920	.0335709
			3	.24396704	.23491657	.566	-.3733356	.8612697
		3	1	-.8796276*	.23466705	.006	-1.4962291	-.2630260
			2	-.24396704	.23491657	.566	-.8612697	.3733356

*. The mean difference is significant at the 0.05 level.

Lampiran 6. Jurnal Publikasi

Simvastatin Toxicity Induces Mitochondrial Dysfunction in Rat Skeletal Muscle

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ABSTRACT

Background: Statins are the class of drugs that are widely used for lowering LDL cholesterol and as primary and secondary prevention to cardiovascular disease. However, the widespread use of statins is constrained by the presence of toxicity or intolerance, which affects drug control rates. The toxicity or intolerance of statins ranges from 10-15%. The most common statin toxicity is statin-associated muscle symptoms (SAMS). The underlying mechanisms of SAMS involve the disruption of mitochondrial biogenesis, potential membrane changes, reduced number of mitochondria, and changes in protein oxidative activity due to the accumulation of ROS in cells and tissues. The disruption of mitochondrial biogenesis can be marked by a decrease of peroxisome proliferator-activated receptor co-activator gamma (PGC-1α). This study aimed to determine the effect of simvastatin on skeletal muscle PGC-1α.
Methods: Sixteen female Wistar rats (8-10 weeks of age) were randomized into 2 groups: (1) control group (n=8), and (2) simvastatin group(n=8). For 30 days, the simvastatin group was exposed to simvastatin at a dose of 10 mg/kg/day. Meanwhile, the control group animals only received 0.5% methyl cellulose. Gastrocnemius muscles were collected and PGC-1α levels were evaluated by using ELISA Kit.
Results: Following 30 days of treatment, a significantly lower level of skeletal muscle PGC-1αwas observed in the simvastatin group compared to the control group (p = .026).
Conclusion: Our finding indicates that administration of simvastatin at a dose of 10 mg/kg/day for 30 days may decrease skeletal muscle PGC-1α leading to mitochondrial dysfunction in rat skeletal muscle.
Keywords: Statin; Toxicity; Mitochondrial dysfunction; peroxisome proliferator-activated receptor co-activator gamma; Skeletal muscle

INTRODUCTION

Statins are the class of drugs that are widely used for lowering LDL cholesterol and as primary and secondary prevention to cardiovascular disease [(1)]. The widespread use of statins is restricted by the presence of toxicity or the associated intolerance, which influences the rates of drug monitoring. Statin toxicity or intolerance varies from 10 to 15%. In other studies, toxicity can approach 30 % [(2)]. Statin-associated muscle symptoms have been the most common statin toxicity (SAMS). The underlying mechanisms of SAMS involve the disruption of mitochondrial biogenesis, potential membrane changes, reduced number of mitochondria, and changes in protein oxidative activity due to the accumulation of ROS in cells and tissues. The disruption of mitochondrial biogenesis can be marked by a decrease of peroxisome proliferator-activated receptor co-activator gamma (PGC-1α). This study aimed to determine the effect of simvastatin on skeletal muscle PGC-1α.

MATERIAL AND METHOD

Animal

Sixteen female Wistar rats (8-10 weeks of age), were housed in proportion 3 rats per cage with a 12h light/dark cycle. The sample size was determined by using *the Research Equation Method*, where E is the total number of animals – total number of groups. The value of E should lie between 10 and 20. Any sample size, which keeps E between 10 and 20, should be considered as adequate. The total number of animals included in this study were 16 rats. The total numbers of groups in this study were 2 groups. [(3)]

Rats were randomized into 2 groups: (1) control group (n=8), (2) simvastatin group (n=8). For 30 days, the simvastatin group was exposed to simvastatin at a dose of 10 mg/kg/day. Simvastatin (Kimia Farma, Indonesia) was suspended in 0.5% methyl cellulose and administered via oral gavage at a dose of 5.0 ml/kg. Meanwhile, control group animals received 0.5% methyl cellulose by oral gavage at the same relative volume for 30 days. Food consumption was

monitored daily and rat body mass was measured every week. On day 30, 24 hours following the last simvastatin or vehicle treatment, animals were sacrificed by intraperitoneal injection of ketamine (0.05 $ml.kg^{-1}$) dan xylazine (0.01 $ml.kg^{-1}$) followed by cervical dislocation. Gastrocnemius tissue was collected and stored immediately at -20°C for further analysis.

Measurement of PGC-1α Concentration

The tissue samples (90-100 mg) were homogenized with 1000 μL ice-cold PBS using ultra-turrax homogenizer. Homogenates were centrifuged for 5 minutes at 5,000 *g* at 4°C. Supernatants were removed and aliquots were stored at -20^0C. Protein contents of the homogenates were quantified using a Thermo fisher Bradford Assay. Quantitative measurement of PGC-1α in tissue homogenate samples was performed by using a commercial enzyme-linked immunosorbent assay (ELISA) kit, according to the manufacturer's instructions. Absorbance from each sample was measured in duplicate using a microplate reader at a wavelength of 450 nm. PGC-1α concentration data (ng/mg) were presented as a ratio between PGC-1α concentration (ng/mL) and total protein content of homogenates (mg/mL).

Statistical Analysis

All measurement data are expressed as mean ± standard error mean (SEM). To determine the effect of simvastatin on skeletal muscle PGC-1α, an independent t-test was performed to identify the differences of skeletal muscle PGC-1α between the control and simvastatin groups. A P value < 0.05 was considered statistically significant. All statistical analyses were performed using the statistical software SPSS version 23.0

RESULT AND DISCUSSION

Table 1 summarizes the changes in the anthropometric profiles in both groups. No significant differences in body weight changes were observed between the control and simvastatin groups. However, the increase in BMI was significantly lower in the simvastatin group compared to the control group ($p = .030$).

Our finding is similar to the study conducted by Seshadri *et al.* (2019) which reported that simvastatin 20 mg $kg^{-1}day^{-1}$ did not provide a significant change in body weight in high-sucrose diet rats following 30 days of administration. However, when a longer duration of simvastatin treatment (i.e up to 80 days) was applied, significant weight loss was observed.[4]

Table 1. Anthropometric Changes in Control and Simvastatin Group

Variables	Control (n=8)	Simvastatin Group (n=8)	*p* value
% Body Weight Changes	12.34 ± 6.37	9.77 ± 9.00	.162
% BMI Changes	21.12 ± 18.26	13.86 ± 22.17	.030*

The main finding of the present study (**Figure 1**) revealed a significantly lower level of skeletal muscle PGC-1α in the simvastatin group compared to the control group (p = .026). This is similar to the study by Goodman *et al*.(2015) which found a decrease in PGC-1α levels in the soleus muscles of mice given simvastatin 60 mg $kg^{-1}day^{-1}$ and simvastatin 80 mg $kg^{-1}day^{-1}$ for 2 weeks.[5] Besides, Boutbir *et al.* (2012)showed that there was a decrease in skeletal muscle PGC-1α level in rats treated with atorvastatin 10 mg/Kg BW compared to the control group. In addition, mice received simvastatin 5 mg $kg^{-1}day^{-1}$ for 3 weeks showed worsened muscle dysfunction and impaired mitochondrial respiration in both oxidative and glycolytic muscle fiber types [7]. Statin-induced reduction in PGC-1α can explain that statin-induced myotoxicity can be associated with the occurrence of mitochondrial dysfunction.

Mitochondrial dysfunction is a condition characterized by impaired mitochondrial biogenesis, changes in membrane potential, reduced number of mitochondria, and changes in protein oxidative activity due to accumulation of ROS in cells and tissues. Mitochondrial dysfunction is also defined as a decrease in the ability of the mitochondria to synthesize high energy compounds such as adenosine 5 'triphosphate.

Various hypotheses explain that myotoxicity due to statins may result in mitochondrial dysfunction. Inhibition of HMG-CoA reductase caused by statins

also results in the decrease in several intermediates of the mevalonic pathway, such as dolichol, prenylated protein, and electron transport chain protein, heme A and ubiquinone (coenzyme Q10, CoQ10), which close the bonds between complex I and II of the electron transport chain in mitochondria. [8–10]

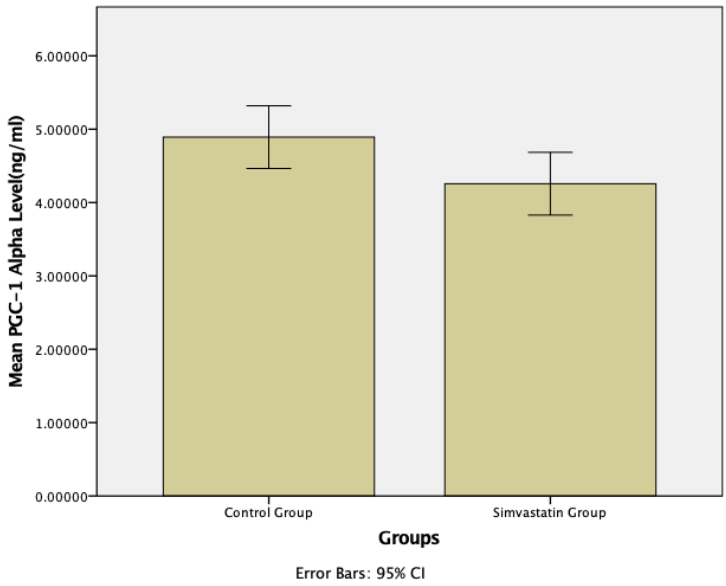

Figure 1. The Differences of Skeletal Muscle *PGC-1α* Levels between Simvastatin and Control Group

CONCLUSION

In conclusion, administration of simvastatin at a dose of 10 mg/kg/day for 30 days may decrease skeletal muscle PGC-1α leading to mitochondrial dysfunction in rat skeletal muscle.

Conflicts of Interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethical Clearance: The Health Research Ethics Committee of the Faculty of Medicine, Hasanuddin University has confirmed the proposal and research protocol (No.458/UN4.6.4.5.31/PP36/2020).

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