



# The Expression of Liver Metabolic Enzymes AMPK $\alpha$ 1, AMPK $\alpha$ 2, and PGC-1 $\alpha$ due to Exercise in Type-2 Diabetes Mellitus Rat Model

Yetty Machrina<sup>1\*</sup>, Yunita Sari Pane<sup>2</sup>, Dharma Lindarto<sup>3</sup>

<sup>1</sup>Department of Physiology, Faculty of Medicine, Universitas Sumatera Utara, Indonesia; <sup>2</sup>Department of Pharmacology, Faculty of Medicine, Universitas Sumatera Utara, Indonesia; <sup>3</sup>Department of Internal Medicine, Endocrinology Division, Faculty of Medicine, Universitas Sumatera Utara, Indonesia

## Abstract

**BACKGROUND:** AMP-activated protein kinase (AMPK) and PGC-1 $\alpha$  were crucial metabolism enzymes not only in the skeletal muscles but also in the liver. Exercise can modify metabolic enzymes to improve insulin resistance.

**AIM:** The aim of this study was to analyze the expression of mRNA liver metabolic enzymes gene, that is, AMPK $\alpha$ 1, AMPK $\alpha$ 2, and PGC-1 $\alpha$  in different types and intensities of exercise.

**METHODS:** Healthy male Wistar rats aged 8 weeks in 150–180 g body weight were given a combination of high fat diet for five weeks and low doses of streptozotocin (30 mg/kgbw and 45 mg/kgbw in 0.1 citrate buffer pH 4,5) to develop type 2 diabetes mellitus (T2DM) rat model. Animals then were divided into five groups: One group was sedentary, and four groups were forced to run on the treadmill 3 times/week, 30 min each session, for 8 weeks. mRNA gene expression of AMPK $\alpha$ 1, AMPK $\alpha$ 2, and PGC-1 $\alpha$  was determined with real-time PCR.

**RESULTS:** The results showed that expression of mRNA AMPK $\alpha$ 1 in treatment groups was elevated than control and the much expression was showed in continuous types. The expression of mRNA AMPK $\alpha$ 2 and PGC-1 $\alpha$  was declined in treatment group which little expression was showed in high intensity for AMPK $\alpha$ 2 and moderate intensity for PGC-1 $\alpha$ . Base on type and exercise intensity, mRNA AMPK $\alpha$ 1 gene expression much in moderate continuous, mRNA AMPK $\alpha$ 2 gene expression higher in high intensity, both continuous and interval training, whereas mRNA PGC-1 $\alpha$  gene expression higher in interval groups.

**CONCLUSION:** Various types of aerobic exercises with moderate-vigorous intensities gave different impact to mRNA liver metabolic enzyme genes.

**Edited by:** Sasho Stoleski  
**Citation:** Machrina Y, Pane YS, Lindarto D. The Expression of Liver Metabolic Enzymes AMPK $\alpha$ 1, AMPK $\alpha$ 2, and PGC-1 $\alpha$  due to Exercise in Type-2 Diabetes Mellitus Rat Model. Open Access Maced J Med Sci. 2020 Sep 30; 8(A):629-632. <https://doi.org/10.3889/oamjms.2020.4550>  
**Keywords:** Aerobic exercise; AMP-activated protein kinase  $\alpha$ 1; AMP-activated protein kinase  $\alpha$ 2; PGC-1 $\alpha$ ; Liver  
**\*Correspondence:** Yetty Machrina, Faculty of medicine, Universitas Sumatera Utara, Jl. Dr. Mansur No.5A, Medan-North Sumatera Utara Province. E-mail: [yetty@usu.ac.id](mailto:yetty@usu.ac.id)  
**Received:** 03-Mar-2020  
**Revised:** 07-Sep-2020  
**Accepted:** 15-Sep-2020  
**Copyright:** © 2020 Yetty Machrina, Yunita Sari Pane, Dharma Lindarto  
**Funding:** This study was supported by a research grant from Universitas Sumatera Utara  
**Competing Interests:** The authors have declared that no competing interests  
**Open Access:** This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

## Introduction

The liver has an important function to maintain blood glucose homeostasis as well as skeletal muscle and adipose tissue in both fed state and fasted state. In the fed state, glucose is stored in the liver as glycogen, while in a fasted state, liver catabolism will produce glucose from glycolysis and gluconeogenesis [1], [2]. Liver metabolism requires enzymes to work properly. One of the most important enzymes to regulate cellular metabolism is AMP-activated protein kinase (AMPK). It is the main regulator of cellular energy and whole-body homeostasis that coordinates several metabolic pathways to adapt cellular processes to energy status [3], [4], [5].

Every physiological and pathological process that changes AMP/ATP ratio due to excessive use of ATP or lack of ATP production will activate AMPK. Activated AMPK switches cells metabolism from anabolic to catabolic state, in fed state to fasted state, initiating an ATP producing pathway to restore energy balance. AMPK phosphorylates the transcriptional coactivator peroxisome proliferator-activated receptor coactivator-1 $\alpha$  (PGC-1 $\alpha$ ), which controls the expression of multiple

transcription factors to induce mitochondrial biogenesis [6]. Phosphorylating results in greater mitochondrial oxidative capacity by increasing PGC-1 expression and activation through PGC-1 promoter autoregulation. This process can lead to glucose transporter activation [7].

Several studies found that metabolic disorder due to mitochondrial dysfunction caused by mitochondrial biogenesis decreasing promote insulin resistance. Low hepatic PGC1- $\alpha$  levels are associated with insulin resistance. PGC-1 $\alpha$  level determines the relative ratio of IRS1 and IRS2 in hepatocytes insulin resistance in the liver disrupt the glycogenesis process and increase gluconeogenesis as compensation mechanism of cell requirement for glucose [8], [9], [10], [11].

Exercise recently is known to improve insulin sensitivity, by enhancing AMPK and PGC-1 $\alpha$  activity in mitochondria. A previous study shown that insulin receptor expression of the skeletal muscle upregulated after 8 weeks of exercise followed by decreasing insulin resistance at various type of exercise [12]. Increased insulin sensitivity in the muscles, thereby reducing insulin resistance, should be followed by metabolic changes in the liver. Hence, this study aimed to find out the expression of AMPK $\alpha$ 1, AMPK $\alpha$ 2, and PGC-1 $\alpha$  in

type 2 diabetes mellitus (T2DM) rat model liver due to different types of exercise with different intensities.

## Methods

### Animal model

The experimental animals used in this study were adult male Wistar (*Rattus norvegicus*, sp) which obtained from Bogor agricultural institute with an estimated body weight of 150–180 g. For 7 days, rats were given standard food as chow and drank *ad libitum*. To develop a T2DM rat model, all rats were given a high-fat diet on chow for 5 weeks with a composition of 41% fat, 41% carbohydrate, and 18% protein. Food weight and the cast-off weighed every day to determine the adequacy of the experimental animal calories. After 5 weeks of administration, rats were injected with a 30 mg/kgbw and 45 mg/kgbw of streptozotocin in 0.1 citrate buffer pH 4,5 at a 1-week interval. Examination of fasting blood sugar was carried out after rats were fasted for 10–12 h to ensure whether rat has become a T2DM model.

### Intervention, blood collection, and blood glucose examination

T2DM rat randomly separates into five groups; sedentary, continuous training in moderate-intensity and high-intensity, slow- and fast-interval training. All the T2DM rats except sedentary group get used to run on the treadmill before protocol exercise was begun in 15 m/min speed for 5 days. The exercise was held 3 times a week for 2 months. Exercise protocol for T2DM is shown in Table 1.

**Table 1: Exercise model and exercise intensity**

Exercise model and intensity	Speed
Moderate intensity continuous training (P1)	25 m/min, for 30 min
High-intensity continuous training (P2)	30 m/min, for 30 min
Slow interval training (P3)	25 m/min, 2 min × 10, with 1 min active rest
Fast interval training (P4)	30 s × 15, with 1 min active rest

After all, the protocol has assigned, T2DM fasted 10–12 h. Lateral vein at the tail was anaesthetized by xylocaine spray. Blood was taken from a vein as much as 3 ml and collected in a non-EDTA tube, then centrifuged in 6000 rpm. Serum from centrifuge product was assessed for fasting blood glucose level by spectrophotometer.

### Tissue extraction and real-time procedure

The liver was extracted from the experimental animal under sedation. Sixty milligrams of liver tissue were inserted into RNA later and stored into the refrigerator –800°C. RNA from 20–30 mg tissue liver was isolated using the RNA easy Mini Kit (Qiagen,

Cat. Nos .74104 dan 74106). Master mix Sybr Green RT-PCR two-step was used to make the working solution as well as gene primer of AMPK  $\alpha$ 1, AMPK  $\alpha$ 2, and PGC-1  $\alpha$ . In this study, beta-actin was chosen as a determinant gene. We used Rotor gene (Qiagen) to asses mRNA gene expression.

Gene name	Primer
AMPK $\alpha$ 1	Forward 5'-ATCCGCAGAGATCCAGAA-3'
	Reverse 5'-CGTCGACTCTCCTTTTCGTC-3'
AMPK $\alpha$ 2	Forward 5'-GCTGTGGATCGCCAAATTAT-3'
	Reverse 5'-GCATCAGCAGAGTGCAATA-3'
PGC-1 $\alpha$	Forward 5'-CACCGTAAATCTGCGGGATG-3'
	Reverse 5'-TATCCATTCTCAAGAGCAGCGAAAAG-3'
Beta-actin	Forward 5'- CCGTAAAGACCTCTATGCCAACA-3'
	Reverse 5'-GCTAGGAGCCAGGGCAGTAATC-3'

### Data analysis

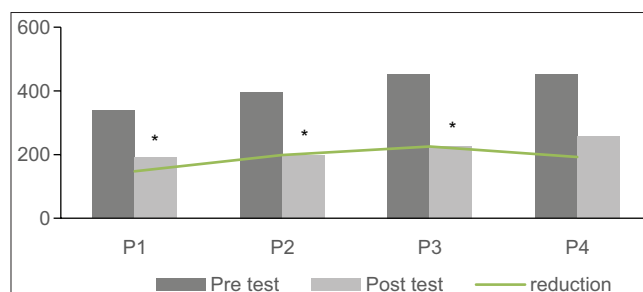
The different blood glucose level pre-test and post-test were analyzed with *independent t-test*. Data were analyzed using SPSS program, it significant if p-value <0,05. Expression of mRNA each gene was described in a mean of cycling threshold, whenever up-regulate or down-regulate expression compared control determined with livask method.

### Ethical approval

Animal handling and intervention procedure have been approved by the Local Ethical Committee on Faculty of Medicine, Universitas Sumatera Utara.

## Results

From this study, we found that fasting blood glucose reduced after 8 weeks of exercise. Fasting blood glucose pre-test and post-test are shown in Figure 1.



**Figure 1: Fasting blood glucose treatment groups before and after exercise protocol. P1=moderate continuous training, P2=severe continuous training, P3=slow interval training, P4=fast interval training. (\*)p < 0.05**

Figure 1 showed that all exercise models can reduce blood glucose level significantly, and the highest reduction found in slow interval training.

To know the expression of mRNA gene AMPK $\alpha$ 1, AMPK $\alpha$ 2 and PGC-1 $\alpha$ , cycling threshold from each gene was recorded, and the expression was assessed by livask method.

Table 2 shows that there was shorter amplification for AMPK $\alpha$ 1 gene, and longer amplification for AMPK $\alpha$ 2 gene and PGC-1 $\alpha$  gene in the exercise group compared with control. It means that there were increasing of AMPK $\alpha$ 1 gene expression and

decreasing of AMPK $\alpha$ 2 and PGC-1 $\alpha$  gene expression after 8-week exercise protocol (Figure 2). Expression of AMPK $\alpha$ 1, AMPK $\alpha$ 2, and PGC-1 $\alpha$  gene were different in each model of exercise with different intensity between moderate continuous training and severe continuous training, slow interval training, and fast interval training.

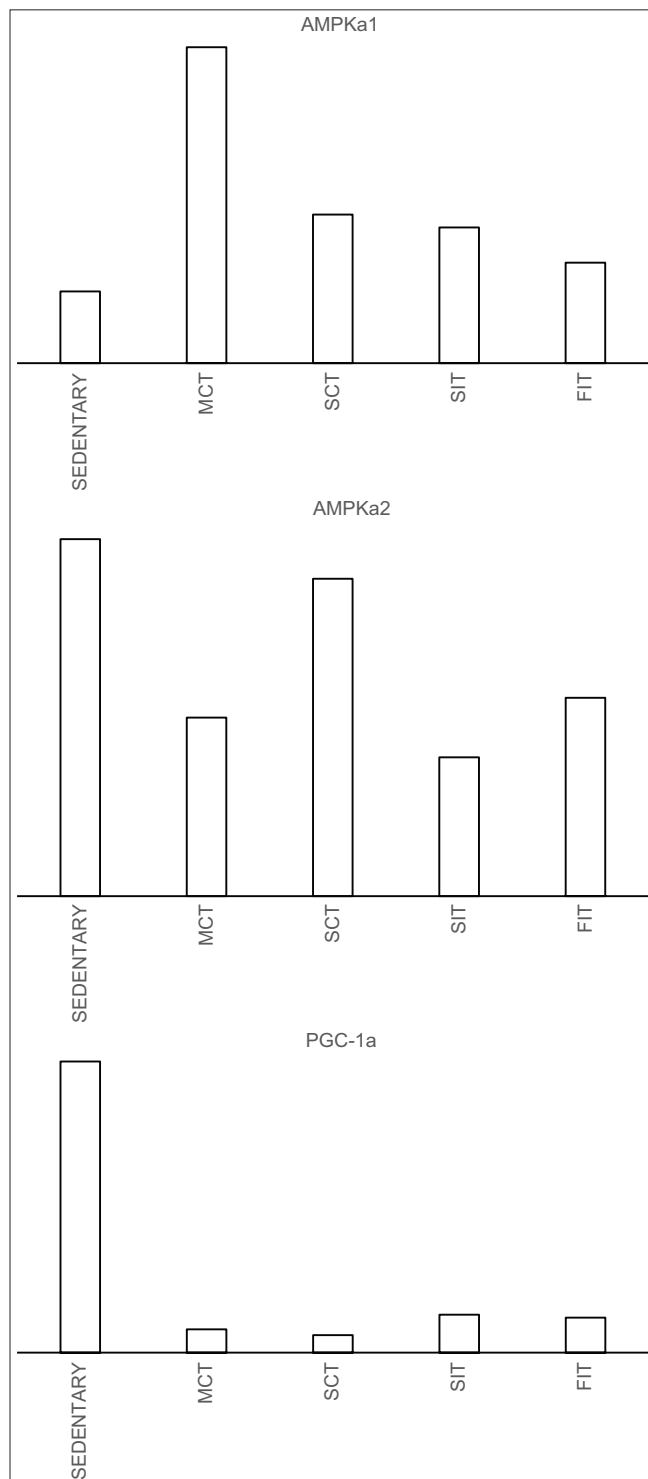


Figure 2: The expression of AMPK $\alpha$ 1, AMPK  $\alpha$ 2, and PGC-1  $\alpha$  in various exercise models

## Discussion

This study aimed to determine the expression of AMPK $\alpha$ 1, AMPK  $\alpha$ 2, and PGC-1  $\alpha$  gene in T2DM rat liver after exercise assignment. The results found that liver metabolic enzyme expression, that is, AMPK $\alpha$ 1, AMPK $\alpha$ 2, and PGC-1 $\alpha$  mitochondria in each model of exercise which arrange every 2 days a week for 8 weeks showed the differences. In this study, fasting blood glucose level significantly declined at moderate continuous training, severe continuous training, and slow interval training even fast interval training. Decreasing in blood glucose as a result of exercise will affect the liver working maintain glucose homeostasis. AMPK $\alpha$ 1 gene expression found upregulation compare sedentary group; meanwhile, AMPK $\alpha$ 2 gene and PGC-1 $\alpha$  gene expression showed downregulation after 8-week exercise. This is such as Rohling, which found that AMPK has an important rule to improve insulin resistance in exercise [13].

AMP-activated Protein Kinase (AMPK) known as mitochondrial metabolic enzyme monitoring systemic and cellular energy to protect the cell from deficit energy status. which promote gluconeogenesis to produce glucose [14], [5] AMPK subunit  $\alpha$  was catalytic but the specific role of AMPK $\alpha$ 1 and AMPK $\alpha$ 2 in metabolism still unclear. AMPK $\alpha$ 1 is in cytoplasm and membrane cell [15], while AMPK $\alpha$ 2 binds to regulatory  $\beta$ 2 subunit. Foretz reported that long-term condition of AMPK $\alpha$ 2 could enhance lipid oxidation, reduce fatty liver so insulin resistance in the liver improved [16].

Gene transcription which involves in lipogenesis and mitochondrial biogenesis such as PGC-1 $\alpha$  modulated by AMPK [17]. Activation of AMPK results in the mobilization of nutrient uptake and catabolism for mitochondrial ATP generation to restore energy homeostasis impact of modulating mitochondrial function on nutrient metabolism in multiple tissues and on glucose and lipid homeostasis in diabetic animal models [18]. The current study showed that both AMPK  $\alpha$  2 and PGC-1 $\alpha$  gene expression downregulated compared inactivity T2DM rat model. Weikert and Pfeifer found that there was a strong correlation between AMPK and PGC-1 $\alpha$  liver mitochondria that

Table 2: The expression of mRNA gene AMPK $\alpha$ 1, AMPK $\alpha$ 2, and PGC-1 $\alpha$

Gene	S ( $\Delta$ ct)	P1 ( $\Delta$ ct)	P1/S ( $\Delta\Delta$ ct)	P2 ( $\Delta$ ct)	P2/S ( $\Delta\Delta$ ct)	P3 ( $\Delta$ ct)	P3/S ( $\Delta\Delta$ ct)	P4 ( $\Delta$ ct)	P4/S ( $\Delta\Delta$ ct)
AMPK $\alpha$ 1	12.67	10.54	4.4 fold	11.63	2.07 fold	12.12	1.87 fold	12.19	1.4 fold
AMPK $\alpha$ 2	7.04	7.18	0.91 fold	7.07	0.98 fold	7.21	0.89 fold	7.17	0.92 fold
PGC-1 $\alpha$	6.05	9.62	0.08 fold	8.98	0.06 fold	10.04	0.13 fold	9.07	0.12 fold

have a crucial function to supply blood glucose through gluconeogenesis in T2DM in other cell has energy [19].

Enhancing on insulin sensitivity at the liver, improved insulin resistance, and liver will not produce gluconeogenesis. It indicate that mitochondrial dysfunction in the liver improved. Mitochondrial biogenesis regulates by PGC-1 $\alpha$  [20], whenever insulin resistance has improved and fasting plasma glucose has gone to normal, PGC-1 $\alpha$  as mitochondrial biogenesis regulator does not need to overexpress. That is why PGC-1 $\alpha$  gene expression in the liver at this study downregulate.

## Conclusion

We conclude that exercise could affect to metabolic enzymes gene expression in the liver of T2DM rat model, that is, AMPK $\alpha$ 1, AMPK $\alpha$ 2, and mitochondrial biogenesis through PGC-1 $\alpha$ . Intensity and exercise model seem to influence the expression of genes.

## References

- Han HS, Kang G, Kim JS, Choi BH, Koo SH. Regulation of glucose metabolism from a liver-centric perspective. *Exp Mol Med*. 2016;48(3):e218. <https://doi.org/10.1038/emm.2015.122> PMID:26964834
- Petersen MC, Vatner DF, Shulman GI. Regulation of hepatic glucose metabolism in health and disease. *Nat Rev Endocrinol*. 2017;13(10):572-87. <https://doi.org/10.1038/nrendo.2017.80> PMID:28731034
- Foretz M, Viollet B. Regulation of hepatic metabolism by AMPK. *J Hepatol*. 2011;54(4):827-9. <https://doi.org/10.1016/j.jhep.2010.09.014> PMID:21163246
- Lin SC, Hardie DG. AMPK: Sensing glucose as well as cellular energy status. *Cell Metab*. 2018;27(2):299-313. <https://doi.org/10.1016/j.cmet.2017.10.009> PMID:29153408
- Garcia D, Shaw RJ. AMPK: Mechanisms of cellular energy sensing and restoration of metabolic balance. *Mol Cell*. 2017;66(6):789-800. <https://doi.org/10.1016/j.molcel.2017.05.032> PMID:28622524
- Wu H, Deng X, Shi Y, Su Y, Wei J, Duan H. PGC-1 $\alpha$ , glucose metabolism and Type 2 diabetes mellitus. *J Endocrinol*. 2016;229(3):R99-115. <https://doi.org/10.1530/joe-16-0021> PMID:27094040
- Liang H, Ward WF. PGC-1 $\alpha$ : A key regulator of energy metabolism. *Adv Physiol Educ*. 2006;30(4):145-51. <https://doi.org/10.1152/advan.00052.2006> PMID:17108241
- Besse-Patin A, Jeromson S, Levesque-Damphousse P, Secco B, Laplante M, Estall JL. PGC1A regulates the IRS1:IRS2 ratio during fasting to influence hepatic metabolism downstream of insulin. *Proc Natl Acad Sci*. 2019;116(10):4285-90. <https://doi.org/10.1073/pnas.1815150116>
- Kim JA, Wei Y, Sowers JR. Role of mitochondrial dysfunction in insulin resistance. *Circ Res*. 2008;102(4):401-14. PMID:18309108
- Montgomery MK, Turner N. Mitochondrial dysfunction and insulin resistance: An update. *Endocr Connect*. 2015;4(1):R1-15. <https://doi.org/10.1530/ec-14-0092> PMID:25385852
- Hatting M, Tavares CD, Sharabi K, Rines AK, Puigserver P. Insulin regulation of gluconeogenesis. *Ann N Y Acad Sci*. 2018;1411(1):21-35. <https://doi.org/10.1111/nyas.13435> PMID:28868790
- Machrina Y, Harun AL, Purba A, Lindarto D. Effect various type of exercise to Insr gene expression, skeletal muscle insulin receptor and insulin resistance on diabetes mellitus Type-2 model rats. *Int J Health Sci*. 2018;6(4):50-6.
- Röhling M, Herder C, Stemper T, Müssig K. Influence of acute and chronic exercise on glucose uptake. *J Diabetes Res*. 2016;2016:2868652. <https://doi.org/10.1155/2016/2868652> PMID:27069930
- Hardie DG. AMPK-sensing energy while talking to other signaling pathways. *Cell Metab*. 2014;20(6):939-52. <https://doi.org/10.1016/j.cmet.2014.09.013> PMID:25448702
- Xiao B, Sanders MJ, Carmena D, Bright NJ, Haire LF, Underwood E, *et al*. Structural basis of AMPK regulation by small molecule activators. *Nat Commun*. 2013;4:3017. <https://doi.org/10.1038/ncomms4017> PMID:24352254
- Foretz M, Even P, Viollet B. AMPK activation reduces hepatic lipid content by increasing fat oxidation *in vivo*. *Int J Mol Sci*. 2018;19(9):2826. <https://doi.org/10.3390/ijms19092826> PMID:30235785
- Viollet B, Guigas B, Leclerc J, Hebrard S, Lantier L, Mounier R, *et al*. AMP-activated protein kinase in the regulation of hepatic energy metabolism: From physiology to therapeutic perspectives. *Acta Physiol*. 2009;196(1):81-98. <https://doi.org/10.1111/j.1748-1716.2009.01970.x> PMID:19245656
- Jenkins Y, Sun TQ, Markovtsov V, Foretz M, Li W, Nguyen H, *et al*. AMPK activation through mitochondrial regulation results in increased substrate oxidation and improved metabolic parameters in models of diabetes. *PLoS One*. 2013;8(12):e81870. <https://doi.org/10.1371/journal.pone.0081870> PMID:24339975
- Weickert MO, Pfeiffer AF. Signalling mechanisms linking hepatic glucose and lipid metabolism. *Diabetologia*. 2006;49(8):1732-41. <https://doi.org/10.1007/s00125-006-0551-6> PMID:16718463
- Fernandez-Marcos PJ, Auwerx J. Regulation of PGC-1 $\alpha$ , a nodal regulator of mitochondrial biogenesis. *Am J Clin Nutr*. 2011;93(4):884S-90. <https://doi.org/10.3945/ajcn.110.001917> PMID:21289221