Effect of Intermittent Fasting on Muscle Glycogen Stores in Rats with High-Calorie Diet

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Abstract

Insulin resistance in skeletal muscle is important since it is normally responsible for most of the insulin-mediated glucose disposal and contains the largest glycogen storage regulated by insulin sensitivity. A high-calorie diet is known to be a risk factor for insulin resistance. Lifestyle modification by regulating caloric intake through intermittent fasting (IF) is recommended to prevent insulin resistance. This study aims to analyze the effect of eight weeks of intermittent fasting on skeletal muscle glycogen stores in rats with a four-week high-calorie diet. A total 24 healthy male Wistar rats were divided into four groups, Ad-libitum with a standard diet (AL-SD); Ad-libitum with a high-calorie diet (AL-HCD); IF with a standard diet (IF-SD); IF with a high-calorie diet (IF-HCD). Muscles were obtained and stained with PAS stain to evaluate glycogen expression based on color intensity. The results of the glycogen expression score were obtained as follows, AL-SD=2.113±0.589; AL-HCD=1.827±0.375; IF-SD=2.109±0.499; IF-HCD=1.979±0.334. Although there was no significant difference between groups ($p > 0.05$), IF can prevent excessive reduction of glycogen stores in subjects with a HCD compared to the AL-HCD group. In conclusion, this study suggests that IF might improve the reduction of glycogen stores in muscles due to a high-calorie diet.

Keywords: Intermittent Fasting; Muscle Glycogen; Rats; High-Calorie Diet; Healthy Lifestyle

1. Introduction

Type 2 Diabetes mellitus is a common metabolic disorder and be a global challenge in the healthcare system. It is estimated that 463 million people aged 20-79 live with diabetes. This number will continue to increase so that by 2045 approximately 700 million people worldwide will be living with diabetes [1]. One of the pathophysiologies in type 2 diabetes mellitus is insulin resistance, which is the inability of insulin-sensitive tissues to respond appropriately to insulin resulting in the disturbance of insulin signaling pathways in the target organ, such as muscle tissue [2,3]. Insulin resistance in skeletal muscle is particularly important since it is normally responsible for more than 75% of all insulin-mediated glucose disposal and contains the largest store of glycogen which is strictly regulated by the influence of insulin sensitivity [4,5] . Insulin resistance in skeletal muscle will affect the process of glucose uptake through GLUT4 and the activation of the main enzymes in glucose phosphorylation and glycogen synthesis [6,7] . As a result, in conditions of insulin resistance, the amount of glycogen synthesized by muscles will decrease [8].

The incidence of insulin resistance is indirectly related to an unhealthy lifestyle, such as excessive calorie consumption. In a previous study by Herawati et al. [9] , mice that received a high-glucose diet experienced a higher increase in blood sugar levels than the control group and had a lower density of beta cells, resulting in a decrease in serum insulin levels. Low serum insulin levels cause reduced glucose uptake into cells. The high increase in blood sugar due to high carbohydrate consumption will induce inflammation and insulin resistance so that the efficiency of insulin signal transmission decreases [10]. Therefore, increasing caloric intake can be a risk factor for insulin resistance [11].

Lifestyle modification has been recommended as the primary prevention of insulin resistance, especially the progression to type 2 diabetes mellitus [12–14]. Regulation of caloric intake through intermittent fasting can reduce the risk of insulin resistance. Fasting may represent a possible approach to prevent and minimize disturbances in glucose homeostasis [15]. Another study states that fasting increases the sensitivity of insulin receptor signaling so that it is more ready to stimulate glucose uptake by muscle cells [16,17]. The aim of this study was to investigate the effect of eight weeks of intermittent fasting on muscle glycogen stores given a high-calorie diet for four weeks.

2. Material and Method

Approval for this research has been obtained from the Committee of Health Research, Faculty of Medicine, Universitas Airlangga (No. 63/EC/KEPK/FKUA/2022)

2.1. Animal

The animal subjects were obtained from the Faculty of Veterinary, Universitas Airlangga. The rats were distributed into four groups: (1) Ad-libitum with a standard diet (AL-SD); (2) Ad-libitum with a high-calorie diet (AL-HCD); (3) Intermittent Fasting with a standard diet (IF-SD); (4) Intermittent fasting with a high-calorie diet (IF-HCD). Each group consists of six rats. Acclimatization was carried out for 14 days before the intervention by giving a standard diet and drinking water ad libitum.

The standard diet given to each rat consisted of 75% carbohydrates, 20% proteins, 5% fats, fiber, vitamins, and minerals. The food is served in the form of pellets of 15-20 grams/day. Meanwhile, the high-calorie diet was given a standard diet added with daily oral gavage of glucose solution 0.013 g/g of body weight [9]. IF regimen used in this study was IF 5:2. Every two days a week (Monday and Thursday), rats were fasted for 14 hours (16.00-06.00) by limiting access to food and water. IF groups also received a 20% calorie restriction from the standard diet [15].

For the first four weeks, all rats received a standard diet, with fasting for the IF group and without fasting for the SD group. At the end of the fourth week, AL-SD and IF-SD groups were sacrificed. The remaining two groups (AL-HC and IF-HC) continued the intervention for the next four weeks. At this time, a high-calorie diet was given. At the end of the eighth week, rats were sacrificed.

2.2. Tissue Collecting and Staining

After undergoing the intervention, all rats were sacrificed by anesthetizing using 30% chloroform. The gastrocnemius muscle of the rats was dissected. Portions of the tissue were fixed in a 10% formalin buffer solution and cut into 5-µm-thick paraffin sections. These sections were stained in periodic acid-Schiff (PAS) to detect and quantify the glycogen content. Briefly, 5 µm cross muscle sections were incubated for 10 minutes at room temperature in 0.5% periodic acid, then washed and incubated with Schiff's reagent for 15 minutes.

2.3. Muscle Glycogen Expression Scoring

PAS-stained muscle slides were evaluated using an Olympus photomicroscope. Glycogen expression in PAS-positive cells was observed in 5 fields of view with 200x magnification. The intensity of PAS staining assessed semiquantitatively and graded as: 0 = no staining intensity, 1 = low staining intensity, 2 = moderate staining intensity, and 3 = high staining intensity [18]. Each cell was scored per field of view. Then the average score for each field of view was determined by adding the total score divided by the number of cells per field of view. The final mean score was obtained from the average of 5 fields of view per treatment group.

2.4. Statistical Analysis

The data are expressed as the mean ± SD. Normality and homogeneity test was performed to evaluate data distribution. The data were statistically analyzed by one-way ANOVA (SPSS software 23.0 version for Windows). The results were considered statistically significant when the P value was less than 5%.

3. Result

The data of the variable measured in this study can be seen in the table below.

Table 1 Mean score of muscle glycogen expression in each group

Group	Score (Mean ± SD)
AL-SD	2.113 ± 0.589
AL-HC	1.827 ± 0.375
IF-SD	2.109 ± 0.499
IF-HC	1.979 ± 0.334

The score of muscle glycogen expression did not show a significant difference among groups (P=0.675). However, the average patterns seemed to have a decreasing score trend in the HC group compared with the AL group (figure 1).

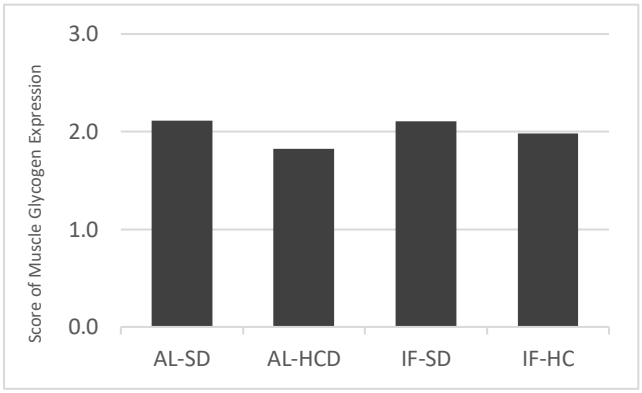

Figure 1 Muscle glycogen expression after treatment. Demonstrated no significantly different (P≥0.05) between groups.

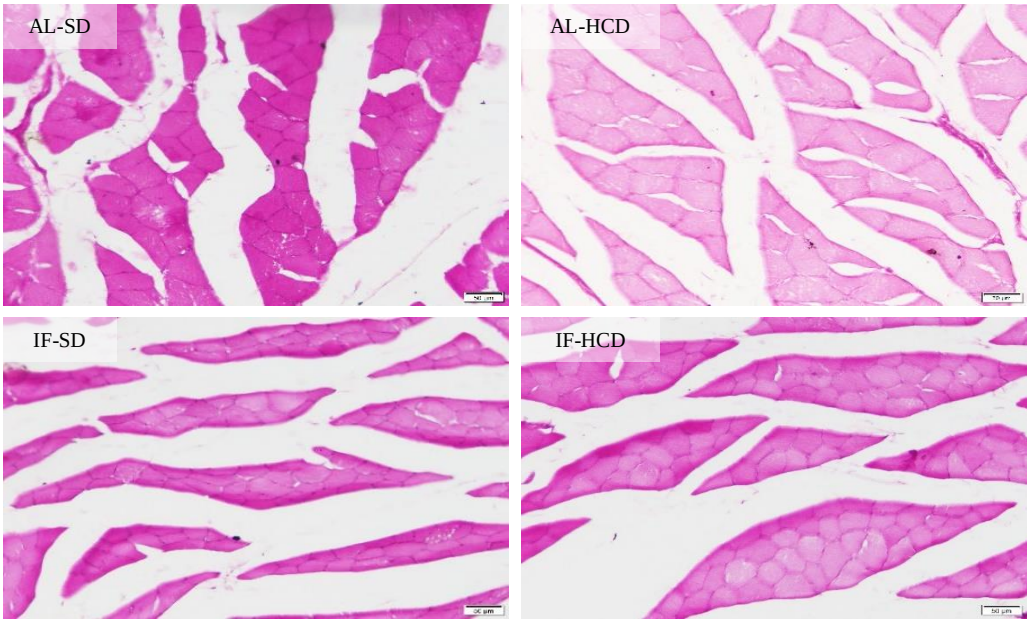

Figure 2 Photomicrograph representative of each group
(a) AL-SD, the non-fasting group without a high-calorie diet); (b) AL-HCD, the non-fasting group with a high-calorie diet; (c) IF-SD is the fasting group without a high-calorie diet; (d) IF-HCD, the fasting group with a high-calorie diet. (PAS staining; 200x magnification; using microscope Olympus, Olympus CellSense)

4. Discussion

The present study demonstrated that intermittent fasting had an insignificant effect on skeletal muscle glycogen stores. However, there was a trend in the IF-SD group to have a lower score than the AL-SD control group. It means that the IF-SD group has lower glycogen stores than the AL-SD group. It is probably caused by changes in body physiology in fasting conditions.

In healthy individuals, after eating, insulin is secreted by pancreatic beta cells, which is stimulated by an increase in blood glucose levels. Insulin will further regulate the process of glucose storage in the liver and muscles in the form of glycogen. Conversely, in fasting conditions, the body does not receive food intake for a certain period. As a result, blood glucose levels tend to fall, which causes a decrease in insulin secretion, and at the same time, the levels of glucagon hormones and catecholamines increase [19].

Other studies have shown a decrease in plasma insulin levels in fasting conditions, which is influenced by the length of fasting; longer fasting duration results in significantly lower plasma insulin levels [20]. This is consistent with previous research by Ayala et al. [21], who showed that 18 hours-fasted rats had significantly lower plasma insulin than 5 hours-fasted rats. The study also found that Gastrocnemius muscle glycogen did not differ significantly between 5 hours-fasted rats and 18 hours-fasted rats, although rats that fasted for 18 hours had lower muscle glycogen levels. Another study by Kokubun et al. [22] showed a decrease in glycogen in rats with fasting intervention for 12 and 48 hours due to a decrease in the amount of activated Akt in skeletal muscle. Akt activation in skeletal muscle cells is stimulated by insulin through a signaling pathway. Insulin will bind to the insulin receptor and autophosphorylate IRS-1, which will activate Akt/PKB. Activation of Akt causes translocation of GLUT4 and results in glucose uptake into cells [23,24].

The lowest average muscle glycogen expression score was found in the AL-HCD group, indicating that the AL-HCD group had fewer glycogen stores among all groups. In this study, a high-calorie diet was given in the form of glucose solution as much as 0.013 gram/gram body weight, equivalent to an additional calorie of 3-5% per day from standard calorie needs. A previous study [8] stated that groups of rats with additional calories in the form of glucose with a high glycemic index had lower serum insulin levels. Low insulin levels cause less glucose to enter the cells. Another study found that a high-carbohydrate diet impaired GLUT4 expression and translocation to the plasma membrane [25].

The insulin signaling pathway in skeletal muscle is an essential process in the normal mechanism of insulin action. Defects in transduction or phosphorylation between signaling molecules such as PI3K, AKT, and GLUT4 are associated with the occurrence of insulin resistance in the development of diabetes and the administration of high-calorie diets [26]. Thus, the lower glycogen stores in the high-calorie diet group may be due to low insulin levels and the development of insulin resistance in skeletal muscle cells.

In addition, this study found that the group that received a high-calorie diet intervention (HCD) showed the fasting group (IF-HCD) had a higher score than the non-fasting group (AL-HCD). It can hypothesize that fasting has an ameliorative effect on insulin resistance induced by a high-calorie diet. From previous studies, fasting is known to be beneficial in preventing and improving aspects of metabolic syndrome in both experimental animals and humans. Intermittent fasting with an ADF regimen for 28 days relieves insulin resistance, prevents weight gain in diabetic rats, increases glycogen synthesis in muscle and liver, activates IRS-1/PKB signaling, and reduces inflammation [27]. Intermittent fasting using the eTRF (early time-restricted feeding) method for five weeks with a 6-hour eating period improved insulin levels, insulin sensitivity, and oxidative stress levels in prediabetic subjects [28]. Subjects with excess body weight who did intermittent fasting 5:2 for six months with calorie consumption of 500-600 calories on fasting days experienced decreased body fat, increased insulin sensitivity, and reduced blood pressure [29]. Ramadan fasting in subjects with metabolic syndrome showed reduced energy intake, decreased plasma glucose levels, and increased insulin sensitivity [30].

5. Conclusion

Our study revealed that intermittent fasting did not show a significant effect on rat muscle glycogen stores. However, intermittent fasting in the present study reduced the decrease in glycogen stores in rats on a high-calorie diet. May intermittent fasting has an ameliorative effect on insulin resistance in muscle cells due to high-calorie

administration. Further research is needed, particularly to understand the mechanism of how fasting can affect muscle glycogen stores in subjects receiving high-calorie diets.

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References

[1] International Diabetes Federation. IDF Diabetes Atlas. Ninth Edit. 2019.

[2] Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al. Molecular Sciences Pathophysiology of Type 2 Diabetes Mellitus. Int J Mol Sci. 2020;21:1–34.

[3] Belwal T, Fazel Nabavi S, Nabavi SM, Habtemariam S. Dietary Anthocyanins and Insulin Resistance: When Food Becomes a Medicine. Nutrients. 2017;9:1–22.

[4] Wei Y, Chen K, Whaley-Connell AT, Stump CS, Ibdah JA, Sowers JR. Skeletal muscle insulin resistance: Role of inflammatory cytokines and reactive oxygen species. Am J Physiol - Regul Integr Comp Physiol. 2008;294(3):R673–80.

[5] He J, Kelley DE. Muscle glycogen content in type 2 diabetes mellitus. Am J Physiol - Endocrinol Metab. 2004;287:1002–7.

[6] Abdul-Ghani MA, Defronzo RA. Pathogenesis of insulin resistance in skeletal muscle. J Biomed Biotechnol. 2010;2010.

[7] Adelia H, Bambang P, Arifa M. The Effect of Eccentric Activity on Glucose Transporter Type 4 in Gastrocnemius Muscle of Streptozot. J Agromedicine Med Sci. 2017;3(3):39–43.

[8] Suarsana IN, Priosoeryanto BP, Wresdiyati T, Bintang M. Sintesis Glikogen Hati dan Otot pada Tikus Diabetes yang Diberi Ekstrak Tempe. J Vet. 2010;11(3):190–5.

[9] Herawati L, Sari GM, Irawan R. High glycemic index diet decreases insulin secretion without altering Akt and Pdx1 expression on pancreatic beta cells in mice. Chiang Mai Univ J Nat Sci. 2020;19(3):366–78.

[10] Hotamisligil GS. Inflammation, metaflammation and immunometabolic disorders. Nat Publ Gr. 2017;542(7640):177–85.

[11] Boden G, Homko C, Barrero CA, Stein PT, Chen X, Cheung P, et al. Excessive caloric intake acutely causes oxidative stress, GLUT4 carbonylation, and insulin resistance in healthy men. Sci Transl Med. 2015;7(304).

[12] Thaane T, Motala AA, Mckune AJ. Lifestyle modification in the management of insulin resistance states in overweight/obesity: the role of exercise training. J Endocrinol Metab Diabetes South Africa. 2019;24(2):65–9.

[13] García-Molina L, Lewis-Mikhael AM, Riquelme-Gallego B, Cano-Ibáñez N, Oliveras-López MJ, Bueno-Cavanillas A. Improving type 2 diabetes mellitus glycaemic control through lifestyle modification implementing diet intervention: a systematic review and meta-analysis. Eur J Nutr. 2020;59(4):1313–28.

[14] Kim HL, Chung J, Kim KJ, Kim HJ, Seo WW, Jeon KH, et al. Lifestyle Modification in the Management of Metabolic Syndrome: Statement from Korean Society of CardioMetabolic Syndrome (KSCMS). Korean Circ J. 2022;52(2):93–109.

[15] Belkacemi L, Selselet-attou G, Hupkens E, Nguidjoe E, Louchami K, Sener A, et al. Intermittent Fasting Modulation of the Diabetic Syndrome in Streptozotocin-Injected Rats. Int J Endocrinol. 2012;2012:1–12.

[16] Sequea DA, Sharma N, Arias EB, Cartee GD. Calorie Restriction Enhances Insulin-Stimulated Glucose Uptake and Akt Phosphorylation in Both Fast-Twitch and Slow-Twitch Skeletal Muscle of 24-Month-Old Rats. J Gerontol A Biol Sci Med Sci. 2012;67(12):1279–85.

[17] Heijboer AC, Donga E, Voshol PJ, Dang Z, Havekes LM, Romijn JA, et al. Sixteen hours of fasting differentially affects hepatic and muscle insulin sensitivity in mice. J Lipid Res. 2005;46(3):582–8.

[18] Firshman AM, Valberg SJ, Bender JB, Annandale EJ, Hayden DW. Comparison of histopathologic criteria and skeletal muscle fixation techniques for the diagnosis of polysaccharide storage myopathy in horses. Vet Pathol. 2006;43(3):257–69.

[19] Meo SA, Hassan M. Physiological changes during fasting in Ramadan. J Pak Med Assoc. 2015;65(5):S6–13.

[20] Jensen TL, Kiersgaard MK, Sørensen DB, Mikkelsen LF. Fasting of mice: A review. Lab Anim. 2013;47(4):225–40.

[21] Ayala JE, Bracy DP, Mcguinness OP, Wasserman DH. Considerations in the Design of Hyperinsulinemic- Euglycemic Clamps in the Conscious Mouse. Diabetes. 2006;55(October 2005):390–7.

[22] Kokubun E, Hirabara SM, Fiamoncini J, Curi R, Haebisch H. Changes of glycogen content in liver , skeletal muscle , and heart from

fasted rats. Cell Biochem Funct. 2009;27(July):488–95.

[23] Sweeney EL, Jeromson S, Hamilton DL, Brooks NE, Walshe IH. Skeletal muscle insulin signaling and whole-body glucose metabolism following acute sleep restriction in healthy males. 2017;5:1–8.

[24] Petersen MC, Shulman GI. Mechanisms of insulin action and insulin resistance. Physiol Rev. 2018;98(4):2133–223.

[25] Campello RS, Alves-wagner ABT, Abdulkader F, Cristina R, Mori T, Machado UF. Carbohydrate- and lipid-enriched meals acutely disrupt glycemic homeostasis by inducing transient insulin resistance in rats. Can J Physiol Pharmacol. 2012;545(March):537–45.

[26] Zhang L, Huang Y juan, Sun J pan, Zhang T ying, Liu T li, Ke B, et al. Protective effects of calorie restriction on insulin resistance and islet function in STZ-induced type 2 diabetes rats. Nutr Metab [Internet]. 2021;18(1):1–10. Available from: https://doi.org/10.1186/s12986-021-00575-y

[27] Zhang H, Zhang W, Yun D, Li L, Zhao W, Li Y, et al. ScienceDirect Alternate-day fasting alleviates diabetes-induced glycolipid metabolism disorders : roles of FGF21 and bile acids. J Nutr Biochem [Internet]. 2020;83:108403. Available from: https://doi.org/10.1016/j.jnutbio.2020.108403

[28] Sutton EF, Beyl R, Early KS, Cefalu WT, Ravussin E, Peterson CM. Early Time-Restricted Feeding Improves Insulin Sensitivity Even Without Weight Loss in Men with Prediabetes. Cell Metab. 2018;27(6):1212–21.

[29] Harvie MN, Pegington M, Mattson MP, Frystyk J, Dillon B, Evans G, et al. The effects of intermittent or continuous energy restriction on weight loss and metabolic disease risk markers: A randomized trial in young overweight women. Int J Obes [Internet]. 2011;35(5):714–27. Available from: http://dx.doi.org/10.1038/ijo.2010.171

[30] Shariatpanahi VZ, Shariatpanahi VM, Shahbazi S, Hossaini A, Abadi A. Effect of Ramadan fasting on some indices of insulin resistance and components of the metabolic syndrome in healthy male adults. Br J Nutr. 2008;100(1):147–51.